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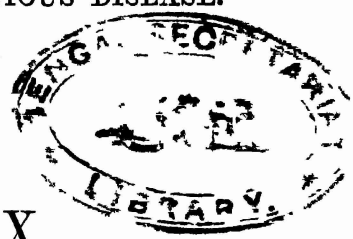

SUBJECT:

ANTHRAX.

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PREFACE.

THIS article has been compiled chiefly from the last edition of Galtier's "Maladies contagieuses," but many additions have been made. The following works have been consulted: Micro-organisms and Disease, Sydenham Society; Koch on Disinfection; Friedberger and Frohner, Medicine; Fleming's Sanitary Science; "Crookshank's Manual of Bacteriology"; Cornil and Babes "Les Bacteries"; Frænkel's Manual of Bacteriology; Armatage, Disease of the Ox; Peuch and Toussaint on the Ox, etc. The subject of Loodianah disease in the horse has not been dealt with, being at present under investigation. The compiler would be greatly obliged for cover glass preparations and blood in capillary tubes, also pieces of tissue (spleen, kidney, etc.) in alcohol, from horses attacked with Loodianah disease.

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MEERUT, 1894.

ANTHRAX.

Synonyms.—Charbon Bacteridien; Sang de rate; Fievre charbonneuse; Splenic apoplexy; Miltzbrand, etc., etc. Until comparatively recent times the greatest confusion appears to have prevailed as to the disease which is properly designated Anthrax. A host of diseases having somewhat similar characters, symptoms, and *post-mortem* appearances were jumbled up together and spoken of as Anthrax or anthracoid diseases, and so great was the confusion which prevailed, that it was next to impossible to gather from the various descriptions given by different authors, what was, and what was not true Anthrax.

All diseases which were characterized by a dark coloration of the blood and the various organs, and all which were accompanied by crepitant tumefactions of a black colour were called Anthrax. M. Chabert in 1790 first restricted the term Charbon (Anthrax) to three morbid conditions which he designated—1, Charbonous Fever, a form characterized by the absence of external tumours; 2, Essential Charbon, or external charbon commencing by the formation of tumours; and 3, Charbon symptomatique, or charbon commencing with fever and accompanied by external tumours. This was a distinct step in the right direction towards a precise designation of the disease, but it was not scientifically exactly true, as later discoveries made in the latter part of the nineteenth century have proved. At the present time we recognize definitely that there are two charbonous diseases, *viz.*:—1, Charbonous Fever; and, 2, charbon symptomatique or emphysematous Anthrax. Charbonous fever and charbon symptomatique are caused by two entirely different poisons, the distinguishing characters, etc., of which have been definitely worked out, which differ entirely from each other, and which are to be studied each under its own heading: Charbon bacteridien, as it is not essentially characterized by black tumours, has retained the name charbonous fever. It presents the symptoms recognized by Chabert and his successors as those of charbonous fever; it is accompanied by black coloration and viscosity of the blood, and very often by tumefaction of the spleen, and it may even be accompanied by the formation of tumours on the surface of the body in certain species. It manifests itself under different forms, according to the mode of penetration of the virus into the

body, and according to the species of animals which it attacks. Thus it is called malignant pustule in man, splenic apoplexy (Sang de rate) in small ruminants, and charbonous fever or simply charbon in the other species.

Definition.—Charbon is an infectious (Mosselman and Lienaux), virulent, and contagious disease, transmissible to animals and to man. It is due to the entrance of a bacillus, the Bacteridie of Davaine, into the organism and its multiplication therein. The malady evinces itself by profound adynamic fever, with more or less stupor. It is very rapidly evolved, and leads to serious and profound alterations of the blood, the plasma of which becomes tinged with the coloring matter of the red corpuscles which communicates a dirty yellow colour to the mucous membranes. The blood becomes broken down, and black in colour. As a result of these changes in the blood, the circulation is impeded and congestions of various organs result, and hence arise petechiæ, ecchymoses, and hæmorrhages into the various organs and tissues of the body.

Anthrax occasions considerable losses in those countries and localities where it has its seat; it attacks every year a large number of individuals, more particularly in certain countries and at certain seasons, but most commonly in summer and autumn. It attacks more particularly the ovine, caprine, and bovine species which are at pasture; sometimes it attacks also solipeds, in which it is a very grave affection, and even also occasionally the porcine species (Galtier, Malad. contagieuses). It generally assumes an enzootic form in localities where it once appears, and in low-lying marshy countries it occasioned in times past, and still continues to cause, great numbers of deaths where preventive inoculation is not practised to prevent its attacks. In these cases it has been known to carry off 10, 15, or 20 per cent. of the flocks of sheep and herds of cattle.

After having made its first appearance and attacked a number of animals, after having persisted for a longer or shorter time, it re-appears usually every year on account of the longevity of its germs and their resistance to atmospheric influences; animals ingest these germs along with their fodder or with the dirt adhering to it. Anthrax, then, is a plague to agriculture in those countries where it exists to any extent. It is an exceptionally grave affection on account of the mortality which it causes, its transmissibility to man, the length of time which the germs retain their virulence in external media, the length of time which an infected place remains dangerous, and the danger attending the utilization of the carcasses as food.

General symptoms.—The symptoms of Anthrax vary somewhat considerably according to the species of animal which is attacked, to

individual peculiarities of constitution, according to the channel by which the virus has gained access to the system, and to the amount of it which has been introduced. These variations will be briefly noted later on.

Charbon is always sudden in its appearance, manifesting itself by grave symptoms, which run a rapid course, and very frequently have a fatal termination; but at times we meet with cases running an apparently abortive course, the sudden and startling symptoms subsiding almost as suddenly as they appeared and the case terminating in recovery. In some cases so sudden is the appearance and so grave the character of the symptoms, and such is the rapidity with which death takes place, that we have been led to confer on it the name of apoplectic Anthrax, charbon foudroyant, and miltzbrand apoplexie in Europe, and in parts of India "Goli-ki-sat." This form of the disease is generally supposed to be caused by the infection of the animal with the "spores" of Anthrax, and Friedberger and Frohner state that it can be induced experimentally by causing an animal to ingest them. In these cases, from the first appearance of any disturbance to the death of the animal, ten minutes or a quarter of an hour only may elapse, and we often find a number of animals left apparently healthy overnight dead in the morning. This form of the disease is most commonly met with amongst sheep and oxen, and at the beginning of an outbreak, the first cases almost always being the most severe. In these cases of apoplectic Anthrax, the animal generally commences to shiver, staggers about, or goes round in a circle, bleeds from the nose and often also from the anus, breathes very rapidly, and finally falls to the ground, where, after a few convulsive kicks, it dies, blood nearly always issuing from the nose and anus after death. These symptoms sometimes also come on whilst the animal is at work or at pasture. Other cases run a much slower course, the symptoms being much less rapidly evolved, and the disease lasting for from 2 to 24 hours.

Following the reception of the virus of the disease into the system we observe the period of incubation, variable in duration, and during which no important symptom has betrayed the serious changes which are occurring at least when the entrance has occurred at a point which is hidden from view, as in cases where Anthrax is spontaneously contracted by animals ingesting the germs with their food at pasture, etc., and at its termination general symptoms of the disease suddenly appear; for, when the virus gains entry by the digestive organs, or by the respiratory tract, the local disorder which it gives rise to is not visible. In other cases, however,—as, for instance, when the germs have been absorbed by the bucco-pharyngeal mucous membrane,—our attention will most probably be attracted to the lymphatic ganglia in the vicinity, and more

particularly the subglossan and subparotidean ganglia. In other cases, again, when the germs have been absorbed by the surface of a wound situated on the exterior of the body, the local disturbance caused at the point of entry is first visible, there being at first, as we shall see later on, local tumefaction more or less appreciable, which may, however, be absent in some species of animals. The local tumefaction is soon followed by swelling of the ganglia in the vicinity of the injury through which the lymph from the inoculated part passes, and this again is followed by general symptoms of the disease.

Once the malady is clearly declared, its symptoms consist in alteration of the blood and the circulatory apparatus, as well as in the functional modifications which are explained by embarrassment of the circulation and by the toxic phenomena to which the germs or their products give rise. The blood is modified in proportion as the duration of the disease is prolonged. Nearly normal at the commencement it becomes progressively richer in leucocytes, more viscous, its globules become more agglutinative and blacker in colour, reddening more imperfectly on contact with the air; it coagulates less readily, giving a soft, black, and diffluent clot; it assumes virulent properties, is inoculable, and gradually more bacilli appear in it. When the blood has undergone these profound modifications, blood-letting, if attempted, gives a mere dribbling flow from the wound, and if practised, always, if anything, accelerates the course of the disease. On microscopical examination of such blood when it is thus entirely altered we observe immobile batonnets, either straight or curved, cylindrical, and of one or two articulations or segments, easily seen in the spaces between the agglutinated blood corpuscles. If the blood is examined at the commencement of the affection before it has undergone in a marked degree the above-mentioned alterations, it shows regular and non-agglutinated globules; the bacilli are not numerous, and a microscopical examination does not always reveal their presence. Given this double character of the blood of animals affected with Anthrax, it is necessary when a microscopical examination of the blood is negative, to inoculate an animal (a rabbit or a guinea-pig) susceptible to the malady, and to observe whether the disease is contracted by it or not.

Coincident with the alterations above mentioned in the blood, the circulation becomes profoundly modified. The cardiac impulse, normal at first, becomes often strong, tumultuous, and precipitous at a given moment, and later feeble, and indeed nearly imperceptible at the end of the disease. The pulse becomes small, feeble, and almost imperceptible; we sometimes observe a venous pulse very manifest at the jugular. The circulation is impeded on account of the abnormal viscosity of the blood,

and also by the obstruction of a more or less great number of capillaries (bacterian embolism); in those regions where the skin is fine and thin it becomes red and congested; the conjunctival mucous membrane, which often presents petechial spots, becomes of a more or less dark red colour; the other mucous membranes also become congested, or of a reddish yellow, blue, purple, or black colour and studded over with ecchymoses, vascular ruptures, hæmorrhages, which give rise to epistaxis, or to the emission of sanguinolent urine or fæces (hæmorrhagic charbon). Œdematous engorgements and tumors make their appearance in the subcutaneous tissues.

At the same time, or even before these disturbances of the circulation, we observe changes in the lymphatics. We observe at first turgescence of the ganglia in the neighbourhood of the point where the virus has been absorbed, and later on, in those through which the lymph is carried by the vessels.

The commencement of the malady is generally accompanied by a notable elevation of the temperature, which rises to 40°, 41°, or 42° C. This fever appears due in part to the reaction of the elements of the organism against the presence of the microbes which they attempt to destroy. We may therefore appreciate the moment of the commencement of Anthrax by the thermometer. This elevation of temperature does not, as a rule, persist for very long, but the normal degree is gradually reached again. When the disease is about to terminate fatally, the temperature often falls below normal, descending towards the end to 36°, 35°, or even 34° C.

Coincident with elevation of the temperature we may observe dryness and staring of the coat, and general hyperæsthesia of the dorsolumbar region. The general sensibility is extremely exaggerated at the commencement, and becomes deadened, more or less, at a later period. We sometimes notice a certain nervous surexcitation at the commencement the animal is restless, excited, cattle bellowing. These may even become rabiform, and we may also have convulsions, spasms, and tetanic convulsions; but to these symptoms signs of dulness, stupefaction, torpor, apathy, and somnolence soon succeed; the strength quickly diminishes, adynamia and ataxy are rapidly produced; the animal becomes weak, totters in its gait, is apathetic, remains plunged in a state of torpor and coma which lasts until death closes the scene. At other times we perceive muscular twitchings of the thighs, shoulders, continuous tossing of the head, shivering, and grinding of the teeth, the eyes revolving in the orbits, etc. These various symptoms are the consequence of poisoning by microbian products, capillary obstruction, embarrassment of the circulation, and localization of the lesions on the nervous centres.

Sometimes symptoms of pulmonary congestion are predominant, the respiration is impeded and accelerated, it increases in rapidity and becomes more and more painful and noisy. In some cases I have noticed it to be rattling, owing to an exudation of serosanguinolent material into the tubes, and in these cases the fluid becoming sucked up into the smaller bronchi occludes them, thus causing death. Occasionally, following a more or less violent congestion of the mucous membranes of the anterior respiratory passages, we get some hæmorrhage from the nose, or the flow of a yellowish red serosity may be noticed issuing from the nostrils.

The digestive apparatus suffers notable modifications. The appetite is diminished, then disappears; thirst is sometimes increased but not always. In cattle rumination ceases, the rumen appears full, the left flank being tense; the digestion is profoundly disturbed.

The tongue and the buccal membrane are in some cases congested, tumefied, and black or violet coloured; the tongue often pendant, cold, and covered at certain points with vesicles filled with sanguinolent serosity. We may also have tumefaction of the throat, more or less easily detected by swelling or by tenderness on handling; this hinders deglutition and respiration, and is a very common form of the disease in India, especially amongst equines (charbonous angina). In these cases the swelling and tumefaction generally commence under the throat and about the parotid region, and extend down the neck and to the chest. This form is said to be due to infection by the bacilli, and it may be produced experimentally, as may all cases of charbon with visible localization (Friedberger and Frohner).

In other cases we observe abdominal symptoms most marked; we have abdominal pains, sometimes very severe, at others more or less fugitive in character. In some we meet with intestinal apoplexy and interrhagia; the fæces in such cases become diarrhœic and sanguinolent as a rule, and on microscopical examination the bacillus may be observed in the blood so mixed with the fæces. Sometimes the rectal mucous membrane is vividly congested, œdematous and everted, appearing as a black or violet tumor at the anus.

The urine often becomes deep brown in colour and even sanguinolent, and in this case it contains the bacilli.

In cattle the milk diminishes in amount, and often becomes red coloured and virulent.

The mucous membrane of the vagina is congested and becomes reddish or black in colour. In certain cases there is more or less manifest congestion of the skin in those regions where it is fine, and we may observe spots or blotches more or less deep black.

Œdematous swellings often occur, either localized or diffuse and more or less extensive, penetrating into the interstices between the muscles, and appearing ordinarily in the neighbourhood of the point of the inoculation or of the absorption, or even at other parts of the body.

In certain cases, subcutaneous tumefactions, more or less voluminous, appear, either as the result of traumatism, or without apparent cause, and these rapidly increase in extent. These tumors appear in various regions—under the arms, throat, in front of the shoulders, prepectoral and prescapular lymphatic glands, under the chest, belly, on the sheath, scrotum, etc., also on the legs. They are at first hot and rather painful, and afterwards become cool. When incised we find them to be formed of a yellow fluid or a semigelatiniform material with a black centre of uncoagulated or partially coagulated blood; they contain bacilli, their appearance is due to congestion of the connective tissue, which is accompanied by exudation, vascular ruptures or hæmorrhages. They may be distinguished from the similar swellings which occur in charbon symptomatique by the act of their being noncrepitant. The form of charbon in which these swellings or œdemata are noticed is that which has been designated "charbon with visible localization;" "carbuncular fever," black variola, etc., and they are observed more particularly in the horse. The duration of the malady varies from three to seven days, and the cases of recovery are more numerous than are those in the other forms.

Forms of Anthrax.—Having given a more or less minute detail of the general symptoms of anthrax in its various forms, I will now give a *résumé* of the divisions adopted by Friedberger and Frohner. They divide the disease firstly into A, Charbon without visible localization; and B, Charbon with visible localization.

A is divided into (1)—**The suracute form,** known by the names of charbonous apoplexy, apoplectic charbon, anthrax acutissimus. The symptomatology is that of cerebral apoplexy. The animal is suddenly attacked, stumbles, and falls to the ground; sanguinolent discharges issue from the mouth and anus. The animal perishes amidst convulsions at the end of a period varying from a few minutes to an hour or more. Animals are very often found dead in the stable. This form is most commonly observed in the sheep and ox, and is most frequently met with at the commencement of outbreaks.

(2) **The acute form.**—The symptoms are evolved more slowly, their duration being from 2 to 24 hours at

the outside. The fever rapidly becomes intense, — 40° to 42° C. or 104° to 107° F. There are sometimes observed symptoms of cerebral hyperæmia, inquietude, excitation, rabiform phenomena, weakness, tottering gait, and lastly death from apoplexy. Sometimes the symptoms of pulmonary congestion predominate; also we may observe sanguinolent discharges from the normal orifices, hæmaturia, petechiæ of the conjunctivæ, convulsions, and in some cases death from asphyxia.

(3) **The sub-acute form.**—The clinical characters resemble those of the acute form are, however, clearer, and run a much slower course—24 hours to 5 or 7 days maximum. There are symptoms due to pulmonary and cerebral congestion. To these manifestations grave intestinal symptoms are often added.

B. Charbon with visible localization.—This form is that in which tumors and cutaneous charbonous œdemata are present, and has been called carbuncular fever. It occurs more particularly in the horse and the ox, but is also seen in the dog. Tumefactions appear on various parts of the body, at first hot and painful; charbonous œdemata are diffuse, doughy, fluctuating, indolent tumefactions. The duration of this form is from 3 to 7 days. Cases in which recovery occurs are more numerous. The fever may occur either before or after the tumefactions. The throat is often affected in this form.

Symptoms of Anthrax in Cattle.—*Apoplectic Anthrax Charbonous fever.*—Charbonus fever may sometimes occur in the large ruminants, and is probably more common in them than it is in the horse. When it does occur, it is, as has been before stated, generally met with at the very commencement of an outbreak of the disease; the first cases seen being those of apoplectic Anthrax, and it is most probably due to the infection having been from the spores, as stated by Friedberger and Frohner. This form appears suddenly without any previous symptom having been manifested, the animals attacked suddenly staggering and falling, bleeding from the nose and mouth, and becoming attacked with convulsions, in the midst of which they die in a very short time. It is not by any means uncommon to find a number of the best animals dead in the yard in the morning, although they were left the previous evening in perfect health apparently. It often, however, announces itself by dullness, loss of appetite, grinding of the teeth, cessation of rumination, weakness, prostration, etc., these being accompanied in some cases by stupor and at others

by great excitability. Trembling, shivering fits and sweats, either general or partial, appear, the loins are tender on pressure. Colic is sometimes observed. The strength rapidly diminishes, the hind quarters swaying about when the animal moves. About this time a fœtid and sanguinolent diarrhœa is often observed. The blood undergoes the changes which have already been indicated. The pulse is frequent, small, and feeble, and the heart-beats at the commencement are often tumultuous, but later on become feeble. The conjunctivæ and gums are injected, sometimes of a yellowish red or rusty colour and dotted over with ecchymoses, at others black or violet coloured. The eyes are sometimes prominent, injected, and tears flow down the face.

The respiration is accelerated, tumultuous, or even panting. The ears and horns are cold, the muffle dry, the mouth becomes cold, the tongue violet or very dark coloured and frequently pendant. Œdemata may or may not appear on the body. Blood or a frothy sanguinolent spume may issue from the nostrils and mouth; it may also appear in the urine and fæces, which are diarrhœic. In a few hours certain of the sick succumb; others resist for a longer time, depending on circumstances, as has already been pointed out. If the symptoms are not intense, they may last for six or seven days, or recovery may take place: when the end comes the animals fall, the abdomen becomes distended, convulsions come on amidst which the animal succumbs. Recovery may occur in a number of cases spontaneously, even in those where no treatment has been adopted.

Glos Anthrax in Cattle.—This form of anthrax with external manifestations is not by any means an uncommon one, and I have had numerous opportunites for observing the disease amongst cattle in India, where it is usually in the northern parts at least called "Galghotu." Cases are constantly occurring in the Punjab, and the fact that it is well known to the zamindars, who describe it perfectly, points to its more or less wide spread.

Glos Anthrax is that form of charbon in which the tongue and throat are more particularly involved, and the supposed reason of this is that the virus gains entry to the system by means of a wound in the mouth. This explanation appears to be a very plausible one, and the more so on account of its being very frequently seen contemporaneously with foot-and-mouth disease, a malady in which the mucous membrane of the mouth and tongue become vesicated and ulcerated, large masses being removed and offering a favourable point of entry to the virus of anthrax.

In this form the mouth and tongue are more particularly the seat of the local manifestations of the disease, which are accompanied by the general symptoms of charbon.

The local symptoms consist in the appearance on the general surfaces of the tongue and the frænum and also on the gums, palate, and lips of an eruption of bullæ or phlyctenæ. These phlyctenæ are white at first, often presenting a pearly aspect, but they soon change to yellow and progressively become almost or entirely black; their situation may be either on the summit of a thickened portion of submucous tissue, or they may be directly under the mucous membrane. It is a very notable fact that the eruption of these vesicles is in the vast majority of instances unaccompanied by fever, and this might perhaps justly be taken as evidence to show that the disease is at first local, and also that it arises from infection through the mouth and does not become general until the vesicles have advanced to maturity, and then the virus enters the general circulation and gives rise to the symptoms of Anthrax fever. This supposition is of very great importance clinically; for if these local symptoms are comparable to what goes on in the point of inoculation in some diseases, we can do much by discharging their contents and subjecting them to stringent disinfection. To resume, the phlyctenæ rapidly increase in size, in some cases assuming the proportions of a fowl's egg. The tongue becomes much inflamed, œdematous, dark in colour and protrudes from the mouth. The vesicles soon become ruptured by the movements of the jaws and tongue, and are replaced by ragged ulcers, red in the centre and black at the circumference. Now appear symptoms of Anthrax fever. The ichorous liquid which flows from these ulcers mixes with the saliva, communicating to it its corrosive properties. This saliva, of a thick, stringy, and sanguinolent appearance, flows from the mouth by both commissures; the tumefaction attacks the lips and cheeks more severely and reaches the base of the tongue, larynx, pharynx, soft palate and parotidean region, so that the animal may die of asphyxia if it has so far resisted the malady. The animal usually succumbs in from 24 to 48 hours after the appearance of the vesicles. The symptoms of this disease shortly then are, the appearance of characteristic phlyctenæ in the mouth without fever generally, tumefaction and inflammation of the parts affected, with difficulty or inability to masticate; maturation of the phlyctenæ, and their rupture, with the appearance of fever and the formation of ulcers, rapid congestion and swelling of the parts, and the invasion of neighbouring parts, consequent difficult respiration, sterterous breathing, etc., hard, rapid pulse at first, but soon becoming feeble; and, in fact, the general disturbance of the circulatory apparatus which are met with in Anthrax.

Symptoms of Anthrax in the Sheep and Goat.—The sheep is very susceptible to the action of the bacillus of Anthrax, and the form which the disease usually takes in that animal is the apoplectic form. It appears suddenly, no prodromic symptoms being witnessed as a rule; it

rapidly terminates in death. The symptoms noticed are that the sheep stops feeding and ruminating, bleats, trembles, the breathing is hurried, the eyes prominent, and visible mucous membranes congested. The animal stumbles about, or perhaps turns round in a circle and falls to the ground; there is much distress, convulsions come on, a frothy sanguinolent discharge issues from the nose, and the urine is mixed with blood; death occurs in an incredibly short time, sometimes in a few moments.

In other cases, however, the disease is announced by certain prodromic symptoms. The animal appears excited, there is unusual expression of countenance, redness of the skin in those parts where it is fine about the eyes, nose, and ears; the conjunctivæ are red and their vessels injected. The temperature is elevated, etc. The blood soon becomes black; the appetite disappears, rumination ceases, the sick animal remains behind the flock, the abdomen becomes distended; the fæces coated, soft, and sanguinolent. The respiration becomes painful, dyspnœic, rattling or whistling; it is accompanied by snorting and a flow of frothy sanguinolent spume from the nose. The urine becomes red and sanguinolent. We may make it flow for inspection by stopping the nostrils for a short time, as recommended by Reynal, or by exercising careful and moderate pressure over the bladder. Tremblings come on; the gait is uncertain and stumbling, and eventually the tottering sufferer falls to the ground, passes blood by the natural orifices, grinds the teeth, is seized with convulsions and cramps; the muscles of the neck and limbs are violently contracted, the head thrown back, the eyes revolve in their sockets, respiration is difficult and accompanied by various sounds; the pulse is small, thready and almost imperceptible; after a few hours of suffering, death occurs in the midst of a tetanic convulsion.

A certain number of sheep which have been either spontaneously or experimentally contaminated escape death after having been more or less gravely sick, but generally the animals attacked succumb unless they belong to certain races, such as Algerian, the receptivity of which is, as we shall see later on, less marked. It has been suggested that the Indian sheep may be endowed with a certain amount of immunity, but this is not by any means the case, as I have been able to destroy them by inoculating them with virulent virus almost invariably.

Symptoms of Anthrax in Soliped Animals.—In the horse, charbonous fever, on the Continent, is often accompanied by colic, severe pains, intestinal apoplexy, enterrhagia, and sometimes by an access of fury, when the disease becomes localized in the nerve centres.

The ordinary symptoms by which the disease announces itself are the following: loss of appetite, elevation of temperature, dulness,

prostration, intermittent colic alternating with somnolence and coma, muscular weakness, swaying of the hind quarters, dryness and heat of the skin; shivering and trembling of the muscles at the arms, limbs, quarters, etc., sometimes vertigo, etc. The blood soon undergoes the changes already noted; the circulation is at first accelerated, the heart-beats are strong and tumultuous, the pulse becomes small, quick, thready, and imperceptible. The mucous membranes are congested, dark coloured, violet, or reddish yellow. The tongue is congested and dark coloured; the colicky pains become more persistent; the animals make frequent expulsive efforts; the rectal mucous membrane is sometimes everted as a violet tumour; the fæces become sanguinolent and diarrhœic. The respiration is precipitous, the expression of the face is pinched; sweats appear; the sick become rapidly weak, and there is some difficulty in maintaining the erect posture; the muscles are agitated by spasmodic movements; the temperature falls lower than normal; the animal falls and dies amidst convulsions. The course of the disease is sometimes very sudden; and in some cases we see hard non-emphysematous and non-crepitant tumours on the surface of the body. On the Continent charbon is an exceedingly grave affection of the horse, chiefly of the young and those which are of sanguine or nervous temperament. The animals succumb rapidly, in a few hours often, generally in less than a day. They nearly always die of this malady. Asses seem to resist it better than horses.

The form of Anthrax occurring in the horse in England is thus described by Gresswell:—This disease, *i.e.*, Anthrax without localization, is rare in Great Britain. The symptoms appear suddenly. The horse has violent muscular tremors, perspires freely, and breathes with difficulty and irregularly. There is complete loss of control of the muscular movements, especially of the lumbar muscles and those of the posterior limbs in particular. The animal staggers and dies convulsed. These acute symptoms, especially those of nervous complications, may, however, abate in two or three days, and finally end in death or recovery. However, the symptoms are not always so rapidly developed. They may begin with a small, weak and dicrotous pulse, uneven surface temperature and violent tumultuous action of the heart. The internal temperature is high, but generally falls before death; the conjunctival membrane is yellowish red and swollen. There is disinclination to move, inco-ordination and loss of power in the muscles of locomotion and twitching of the muscles in various parts. Drowsiness and stupidity with much prostration are marked symptoms. There is a yellowish discharge from the nose, often tinged with blood, and the nasal membrane is covered with blood extravasations. The breath is fœtid and the superficial lymphatic glands swollen; abdominal pain

hown by uneasiness, pawing, and looking to the sides is sometimes manifested. The fæces are fluid or blood stained, the skin is harsh and dry. The respiration becomes tumultuous and hurried, the nostrils dilate, and the animal foams at the mouth. The temperature finally declines, the animal staggers at every step; convulsions, and delirium or coma ensue, and death closes the scene.—(*Gresswell, Manual of Equine Medicine.*)

Symptoms of Charbon in other Animals.—Charbon in the Pig.—Although the pig shows a very slight receptivity to charbon, the affection has sometimes been observed in animals belonging to the porcine species, in France, Germany, Belgium, and England, following the ingestion of charbonous debris: it has also occasionally been transmitted to them by causing them to ingest charbonous material, or by inoculation.

An œdematou sinfiltration is generally produced around the point where the virus has been absorbed. When the disease follows the ingestion of charbonous material, a swelling appears about the throat, due to the infiltration of the tissues about that region, and there are observed, together with the tumefaction of the throat, the characteristic symptoms of charbonous angina, ulceration of the amygdylæ, and extension of the tumefaction towards the face. Charbonous pigs may present the majority of the symptoms observed in herbivora, fever, notable elevation of the temperature, inappetance, general malaise, weakness of the hind quarters, diarrhœa, red spots on the skin, etc. Death may ensue in from one to six days.

Charbon in the Rabbit and Guinea-Pig.—The rabbit and guinea-pig, more especially the latter, have a great receptivity for charbon, which is very easily transmitted to them by the various methods of inoculation, and which generally destroys them very rapidly. Generally the point of inoculation becomes the seat of a very marked infiltration, more especially in the guinea-pig. The rabbit attacked with charbon presents, one or two hours before death, restlessness, and frequently changes his position; the respiration and circulation are accelerated, the animal soon becomes apathetic, insensible, somnolent; there is difficulty in co-ordinating movements, and death soon closes the scene.

Charbon in Man.—Man, although less exposed and less apt to contract charbon than animals, the small ruminants, for instance, may sometimes contract it. He may receive the virus in three ways: by accidental inoculation at the surface of the skin, by ingestion, and by inhalation of the germs. The most common method is by accidental cutaneous inoculation, which gives rise to a local lesion, the "malig-

nant pustule." Sometimes cases of internal charbon, bronchopulmonary or intestinal, show themselves, following the introduction of germs into the digestive or respiratory passages. External charbon, which follows the accidental inoculation of the virus at any point of the surface of the body, is observed in those persons who manipulate fresh charbonous material, or materials containing the spores; it is observed in veterinary surgeons, butchers, horse-slaughterers, herdsmen, etc., and persons who make *post-mortem* examinations of animals which have died from the malady, or who have manipulated the debris: it has also been met with in persons working in wool, hides, horns, etc. Wounds, punctures, excoriations, and scratches which may exist previously or occur at the time of manipulating charbonous materials, may serve as the point of entry of the bacillus or its spores. It may sometimes happen that the contact or the bites of flies which have frequented charbonous carcasses or material may give rise to local charbon.

The bacilli, inoculated into any part of the body whatsoever, multiply, causing at their point of entry a local lesion; then around this lesion a swelling appears, and œdema which is hot and painful and gradually extends. Then the malady becomes general. The germs penetrating the walls of the vessels, or gaining access to the circulation by means of the lymphatic system, are carried to the various organs of the body. The general symptoms which have already appeared, following the development of the local lesion, now become doubled in intensity. The fever becomes very severe, the temperature is elevated, the thirst intense; there is general malaise, headache, and nausea; vomition and diarrhœa occur; then follow prostration, coma, asphyxia, black colour of the skin, convulsions, trismus, tetanus, etc., etc.

We may recognize three successive periods in the evolution of malignant pustule. From the moment of inoculation to the time of the appearence of the first symptom of the disease one, two, three, or sometimes more days pass. The first period is characterized by pricking and itching at the seat of inoculation, soon after which a small phlyctenoid tumour, red at its periphery, and brownish black at the centre, filled with a brown serosity, and soon surrounded or studded around with small fine vesicles, appears. During the second period, the tumor becomes indurated at its base, livid, black, and forms a scab. At the same time the tissues surrounding the eschar become tumified, swollen and œdematous, red or livid in colour, and sometimes covered with small phlyctenæ with reddish-coloured contents. In the third phase, the eschar extends, as does also the peripheric œdema, which invades the arms, neck, trunk, face, etc., and is accompanied by swelling of the

ganglia of the neighbouring parts. The bacilli in greater or less numbers may be seen in the Malphigian layer, the superficial part of the pustule, in the eschar, in the subjacent and peripheral tissues, in the derm and the œdematous subcutaneous tissue, etc.

Man may present gastro-intestinal charbon without any cutaneous lesion. He may contract the malady by ingesting the spores with his food or drink, by ingesting charbonous material in which the spores have been produced and not destroyed again, by ingesting even fresh charbonous material, containing a considerable number of filaments of the virus, etc., etc. The symptoms observed in such cases are as follows :—more or less intense fever, lassitude, abdominal pain, vomition, tympany, sanguinolent diarrhœa, cerebral symptoms, dyspnœa, etc., etc. The bacilli can be demonstrated in the blood.

Lastly, we have observed cases of broncho-pulmonary charbon in persons whose work exposed them to the inhalation of infective dust, notably in wool-workers. The disease manifests itself by the following symptoms :—malaise, general fatigue and somnolence, vertigo ; by coryza, flow of tears from the eyes, cough, difficulty in inspiration, palpitation, by a constricting pain at the base of the thorax, by dyspnœa, expectoration of saliva, signs of pleurisy and broncho-pneumonia; by digestive troubles, nausea, vomition, colic, the appearence of a sanguinolent diarrhœa, etc., etc.

"In fatal cases death may take place in a few hours, or not for five or six days. The duration varies in the different forms. Although if left to itself the disease is almost invariably fatal, many cases of the external form have recovered under suitable treatment."—(*Robert's Medicine.*)

Post-mortem Charges.—The lesions of charbon vary slightly according to the modes of infection, but those which predominate in the majority of cases are alterations of the blood caused by the microbe.

The bodies of animals dead of charbon cool very slowly; they rapidly decompose and become distended with gas. Rigor mortis seldom comes on, blood may be noticed flowing from the mouth and nose, and also from the anus, which may be everted.

The subcutaneous connective tissue presents general congestion more or less marked, and we also find ecchymoses and punctiform hæmorrhages into it; we sometimes meet with greyish serosity, which is, however, generally yellow and gelatiniform ; sometimes even redor sanious and more or less rich in bacilli, according as it is more or less dark coloured and contains hæmorrhagic spots or patches. These infiltrations show themselves more frequently in certain regions, about the ganglia around glands, in the interstices of muscles, and on certain parts such as the throat, neck, etc.

The muscles are congested and spotted with blood spots and hæmorrhages due to embolism of the capillary blood-vessels caused by their becoming blocked up with bacilli. Their tenacity and consistence are diminished and they become softer and more friable. They have a reddish, yellowish, or brownish tint, according to the case.

Tumors are sometimes met with in certain regions in those where the connective tissue is loose and abundant. They may have as their starting point a ganglion, which becomes hypertrophied and congested, and softened and surrounded by a zone of more or less extensive colourless yellow gelatiniform infiltration. They may also be found in regions where there are no ganglia; they offer a central zone formed of black material, and an outer zone yellow and gelatiniform. These tumors are non-crepitant. They have their seat in the subcutaneous connective tissue, they sometimes invade the muscles, which are then black, soft, friable, and surrounded by a more or less diffuse infiltration. The skin is easily detached over the tumors.

The blood offers the most important alterations, and the changes which occur in it are the cause of all the other lesions; it is the blood which carrying the bacilli and their poison spreads these agents through the various tissues and organs of the body. It becomes black even before death; it is generally incoagulable or coagulates badly and imperfectly; it is often muddy or forms soft easily broken-down clots; it is virulent and may be inoculated, producing the disease even before death, and it preserves its virulence for some time after death in the body. The red corpuscles are more or less altered, often irregular in shape and crenated at their margins; they are more sticky, and, adhering to each other, collect into irregular masses. The red corpuscles lose their coloring matter which dissolves in the plasma and by which the lining membrane of the blood-vessels and the heart becomes tinted or stained a cherry-red colour. The blood of animals dead of Anthrax contains the virus in some quantity, and this is most important.

The heart is more or less decolorized and flabby, looking as though it had been cooked, and of an earthy aspect. It is usually filled with black, muddy, and incoagulated blood or blood feebly coagulated into soft black clots. The endocardium is tinted red by the coloring matter of the blood; ecchymoses and hæmorrhages appear on the endocardium, the cardiac muscle, and on the pericardium, which often contains a quantity of red serosity. The vessels contain black blood, viscous, muddy and incoagulable, which gives a red tint to their internal tunics and stains the fingers.

In the capillaries of the various tissues and organs we may meet with clots (Toussaint). The capillary system is more or less gorged

with blood; there is here and there exudation of sanguineous plasma from which results an infiltration of yellow gelatiniform plasma in the tissue around the vessels.

The ganglia are nearly always more or less changed, hypertrophied, congested, infiltrated with fluid, and softened. The ganglia, acting as they do more or less the part of filters, collect as a rule large quantities of bacilli. The ganglia at the seat or point of entry of the bacillus into the system are those first attacked, and as the lymph from them circulates to the neighbouring ganglia, these also become altered. Thus, where the mouth or throat has been the seat of entry of the poison, the ganglia of the throat are first attacked, and we get the enormous swelling of the throat of Loodiana disease, which extends to the ganglia of the neck, and the œdematous swelling then extends down the neck and to the chest.

Generally in the sheep dead of spontaneous charbon we find the ganglia which receive the lymphatics of the tongue and pharynx, as well as those at the commencement of the chest; much altered, and in oxen this is also the case.

The lymphatic vessels, more particularly those in the immediate vicinity of the point of entry of the virus, contain a red-coloured lymph.

The serous membranes are congested, spotted with blood-spots, and often contain a more or less coloured serosity. We frequently meet with more or less marked alterations in the digestive organs.

The mucous membranes of the digestive apparatus may be congested and thickened, and of a blackish or brownish colour. The buccal and pharyngeal mucous membrane, but more particularly the mucous membrane of the tongue, presents this aspect in Glos Anthrax. The tongue in these cases is infiltrated, its vessels contain a muddy, viscous, and incoagulable blood; its epithelium is raised in parts and presents vesicles filled with a black serosity.

The pharyngeal mucous membrane is almost the same, its submucous tissue is strongly congested, hyperæmic, and infiltrated in pharyngeal charbon.

The stomach is in some cases particularly changed, congested, spotted on its mucous and serous surfaces. It sometimes contains liquid tinged with blood. We may find hœmorrhagic infracts on its surface internally, and on scraping the mucous membrance previously cleansed, we see innumerable bacilli.

The peritoneum is often congested, spotted with blood spots and points of hœmorrhage on both its parietal and visceral folds; the capillaries are filled with bacilli and stand out like the veins of a reticulated leaf.

The vessels of the mesentery are filled with muddy blood, and we may find more or less extensive hœmorrhages into it. The fat around the kidneys is often charged with yellow gelatiniform sero-ity which may be deep coloured.

The intestines are frequently changed in a very marked manner, the changes being those of congestion, ecchymoses, and hæmorrhages. The contents of the intestine may be charged with blood of a dirty-black colour. Sometimes we may observe a truly apoplectic condition of the intestinal mucous membrane with a layer of escaped blood at its surface and in its thickness; the tissue of the mucous membrane is then infiltrated with blood or with a sero-sanguinolent fluid.

The liver is earthy, yellow, very friable, and it has the appearance of having been cooked or macerated; its vessels are gorged with black muddy blood, and we may see patches of hæmorrhage on its surface. The bacilli are numerous in the vessels.

The spleen is generally hypertrophied, more particularly in the small ruminants; sometimes, however, it is almost normal, neither tumified, swollen, softened, nor black, and this is more particularly the case when death has come on rapidly, time not having been allowed for the whole organism to become invaded completely. Although we are not warranted in rejecting the idea that Anthrax cannot be present when we find the spleen normal, still at the same time it is none the less true that in many cases the examination of this organ is of great importance in diagnosis. In fact, very frequently the spleen is found to be hypertrophied, tumified, softened, and of a black or dark brown colour. It is sometimes enormously congested and swollen and presenting a very large increase in volume. Sometimes the congestion takes place at certain spots from which results a lumpy appearance of soft tumors, which on incision let escape a black muddy fluid. The bacilli are found in the spleen in enormous quantities.

The kidneys are generally congested; the cortical layer more or less deep coloured, and we find ecchymoses, hæmorrhages, and numerous bacilli. The vesical mucous membrane is congested. The urine becomes brown, sanguinolent, and contains bacilli. This latter condition is more especially met with in the sheep, and is explained by vascular ruptures in the kidneys and the passage of the blood into the urine.

The lungs are more or less congested in parts and present ecchymoses, hæmorrhages, and blood-clots.

The pleura also are congested.

Respiratory mucous membrane is congested, ecchymosed, and covered with a sero-mucous sanguinolent material. Lastly, the lesions of charbon may be found in the nervous centres. The *arachnoid* is sometimes

congested and filled with sero-sanguinolent fluid rich in bacilli. The *pia mater* may be more or less vividly congested, whilst at times may be seen a veritable apoplexy with hæmorrhage at the surface of the brain, the vessels of which are filled with muddy blood. The bacilli are found in the blood in the excreted materials which are sanguinolent, in the lesions of all the tissues and organs: they are more particularly abundant in the capillaries of the tissues or organs which are the seat of the congestions, ecchymoses, spots, etc., in the spleen, ganglia, liver, gastro-intestinal mucous membrane, etc., when these organs are manifestly altered.

Lesions observevd in small Ruminants dead of Splenic Apoplexy.—The lesions generally found in the sheep and goat dead of spontaneous charbon may be stated in the following manner.

Rapid decomposition of the cadaver, which quickly becomes inflated with gas; flow of blood from the mouth, nose, and anus; ecchymoses of the skin, subcutaneous connective tissue, ganglia glands, intestinal mucous membrane, spleen, which is generally hypertrophied, kidneys, respiratory mucous membrane, lung and brain, distension of the capillaries of various organs which are more or less hypertrophied, hæmorrhages on to the respiratory mucous membrane; the digestive mucous membrane, pelvis of the kidney, bladder, and consequent admixture of blood with the excretions; hæmorrhages into the cellular tissue, ganglia, lungs, spleen, kidneys, etc., and alteration of the blood, which is black, tarry, and incoagulable.

The lesions of splenic apoplexy are more disseminated, numerous, and more marked in animals of two or three years of age and in those which are in good condition; they are slighter and less numerous in young animals, old animals, and in those which are in bad condition,—in those, in a word, which have succumbed more rapidly. We find again notable differences in individuals; in such cadavers we find more particularly the spleen and kidneys altered; in others it is the digestive mucous membrane, that of the abomasum and intestine; in others the respiratory organs, etc.

Lesions in Bovines dead of Spontaneous Charbonous Fever.—The lesions of charbonous fever in the large ruminants much resemble those of splenic apoplexy in sheep: rapid decomposition of the cadaver; flow of sanguinolent material from the nose and anus; alteration of the blood, which is black and muddy; congestion, infiltration, ecchymoses, and hæmorrhages into the subcutaneous connective tissue; ganglia in the tissue surrounding the throat, abomasum, intestine; spleen, which is often hypertrophied; kidneys, bladder, serous membranes, etc., etc.

Lesions in Solipeds dead of Spontaneous Charbonous Fever.—In horses dead of charbon we observe, as in the large ruminants, black coloration of the blood, ecchymoses on the various organs, infiltrations, vascular ruptures, and hæmorrhages into the majority of the tissues, the subcutaneous connective tissue, peritoneum, intestinal walls, pericardium, heart, endocardium, ganglia; tumefaction, congestion, and infiltration of the lymphatic system; œdema of various organs and regions, etc., etc.

Causes of charbon.—Charbon is a contagious, virulent, transmissible, inoculable malady, as has been decided by experiment. Its appearance and development are due to a micro-organism which is called the "Bacteridium of Davaine" or the "Bacillus anthracis."

From time immemorial charbon has been seen in various countries as an enzootic affection, and numerous observations have been made at various times of its characters, course, and development. It has been remarked that it resides permanently in certain regions and in certain localities; we have recognized certain places, grazing grounds, as being particularly dangerous, and that the malady ceased its ravages when the flocks or herds were moved to other not dangerous grazing grounds. We have seen the malady exercise its ravages sometimes in very limited regions,—in certain stables, for instance,—without showing itself in the immediate neighbourhood. We have observed it as being more frequent in very wet years, also in hot and humid climates, on marshes, etc. It has been demonstrated that splenic apoplexy is transmissible from sheep to sheep, from sheep to horse, cow, rabbit, etc. That charbonous fever of the horse is transmissible to the horse and from horse to sheep; that the disease in the cow may be given to the sheep and charbonous fever of the horse to the rabbit; also that charbonous fever in animals is identical with malignant pustule in man; that the virulence of the disease does not become lessened by successive transmissions; also that the sheep, rabbit, and horse are more susceptible than the ox; that the virulence may persist for some time after death of the animal; that the disease may be transmitted by transfusion, inoculation, contagion, and exceptionally by cohabitation.

Nature of the Contagium of Charbon.—In the year 1850, Rayer and Davaine observed, for the first time, the presence of batonnets of a particular kind in the blood of charbonous animals. Similar batonnets were afterwards observed and described by Pollender, who ascertained their resistance to the action of acids and bases, and their coloration by iodine; also by Brauell, who observed besides that the blood of the fœtus whose mother had died of charbon, did not possess any virulent properties. The rôle played by these "batonnets" was not demonstrated, nor even

suspected by Davaine, Pollender, and Brauell; but the latter, however had considered their presence as a proof of the existence of charbon Pollender very exactly describe the bacillus of charbon, its length, breadth, and its presence in the disease. He compared it to vibriones, and considered it to belong to the vegetable kingdom (*Cornil and Babes*). Brauell described this bacillus in charbon affecting man (*Virchows Archives, 1857*). Delafond, in the year 1860, in his turn observed the bacillus in charbonous blood, and he had observed it both in the spontaneous and in the inoculated disease. He found them in the blood some hours before death, and he recognized the fact that they become rapidly very abundant during the last moments of life. He found them in considerable numbers in the ganglia adjacent to the point of inoculation; he remarked that the bacilli, imprisoned by the fibrin, become rare in the serum after death, and he indicated the method of finding them in the clot by breaking it up.

Delafond had finally suspected the nature and rôle played by the batonnets discovered by Davaine; he regarded them as vegetable cryptogams, as plantules susceptible to be transformed into mycelium; he tried to cultivate them, and saw that they lengthened, doubling, trebling, and quadrupling their length; he considered them capable of forming spores, a property which did not become experimentally verified until 1876, when Koch accomplished it.

Pasteur having in 1861 demonstrated that butyric fermentation was determined by a bacillus, Davaine returned to his observations of 1850, and asked himself whether the bacillus discovered by him in charbonous blood might not be the pathogenic agent of the malady. He endeavoured to demonstrate its rôle by various experiments. He inoculated rabbits and guinea-pigs with charbonous blood, and took from these inoculated animals, at longer and larger intervals from the time of inoculation, droplets of blood which he studied microscopically, and which he used for further inoculations. In this manner he found that so long as the blood did not contain the bacilli it remained inactive in the production of the disease, but when the bacilli made their appearance in the blood, some hours before death it became virulent and inoculable. These experiments, repeated a number of times, gave the same results, and led to the conclusion that the bacillus, to which he had given the name "Bacteridie," was the agent by which the transmission of the disease was accomplished. Davaine also observed the multiplication of the bacillus in the blood of inoculated animals; he observed that the manifestation of the disease coincided with the multiplication of the bacteridium, and that the blood of inoculated animals only became virulent at the moment when it presented bacilli.

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Davaine had recourse to another method of demonstration; he endeavoured to isolate the bacilli, to obtain blood deprived of them; for this purpose he had recourse to the inoculation of females during the period of gestation. Before him Brauell had observed that the blood of the fœtus, contained in the womb of a charbonous female, did not contain the bacilli, and that, consequently, charbon was not transmissible through the placenta. Davaine, profiting by the knowledge of this fact, inoculated pregnant rabbits and gave them charbon. A comparative examination of the blood of the mother and that of the fœtus revealed the presence of the bacillus in the former and its absence in the latter; the blood of the mother was virulent when inoculated, that of the fœtus was not.

Thanks to these two sets of experiments, Davaine, who had besides found that dried charbonous blood was capable of preserving its virulence, concluded that the bacillus was the sole agent in the genesis and the transmission of charbon.

The observations of Davaine, however, left much to be desired, and gave rise to much discussion for a long time. In the drop of charbonous blood inoculated there existed various elements, such as red corpuscles, white globules, bacilli, granular matter, liquid substances, to which some action might be attributed; and it did not seem correct, under these circumstances, to refer all the changes to the bacillus. In addition to this, not having conceived the mode of reproduction of the bacillus to be by the formation of spores capable of preserving their virulence for a long time, after which they could germinate and reproduce the bacillus and under favourable circumstances determine the same malady, Davaine found it to be impossible to explain certain facts and to reply to the arguments and objections of his adversaries. How, for instance, could it be conceived that a charbonous cadaver was able to preserve its virulence for several weeks, and even a longer time, after death, seeing that the adult virus under the form of the bacillus or rod was not preserved, and could not be found in old carcases? It was impossible to explain how and why charbonous cadavers might remain dangerous for months or even years. Here was a fact, not to be explained by the new theory of Davaine, but which was easily explained later on by the knowledge of the faculty of the bacillus to form very resistant spores.

In the meantime, Davain and Raimbert had met with the bacillus in malignant pustule in man, and had caused charbon by inoculating serum taken from the pustule. But the bacillus continued to be regarded as an epiphenomenon of charbon and not as the morbific agent.

Jaillard and Leplat obtained a malady which killed, in the same manner as charbon, but without bacilli, by inoculating charbonous blood taken from a cadaver in course of putrefaction which did not present bacilli in rods; and they concluded that the rod could not be considered as the morbific agent of charbon. Davaine then distinguished charbon due to rods (bacteridia) and the pretented or so-called charbon without bacteridia, to which he gave the name of "maladie dela vache;" he considered the latter to be a special malady, of a septic nature, killing more quickly than charbon and inoculable to birds.—(*Comptes rendus de l'Academie des Sciences, 1864 to 1873.*)

The facts opposed to the theory of Davaine are easily explained to-day; the bacilli may produce spores under certain conditions, before their destruction in the cadavers. On the other hand, the bacteria of putrefaction, which are found in the digestive passages, very promptly invade the various parts of the cadaver and likewise form spores. Also in examining charbonous blood taken from a cadaver in course of putrefaction, we may observe neither the rods of charbon nor septic bacteria nor vibriones on account of their extreme refractility; in addition to which we do not distinguish, by microscopical examination, the spores of one bacterium from those of another. It occasionally happens that the germs of charbon and septic germs are inoculated together; it is generally septicæmia which develops under these circumstances, and consequently the rods of charbon do not appear in the blood. If the spores develop, producing charbon, we obtain the bacilli without having inoculated the rods. If, then, charbonous cadavers remain virulent for a long time in the soil, it is because the bacilli, before disappearing, form spores which are very resistant. In addition to this, it is not only the charbonous blood which becomes rapidly septic after death; that of animals dead of other diseases becomes so after a few hours.

Brauell considered the bacillus as the diagnostic criterium of charbon, but he did not regard it as the cause of the malady; he recognized in 1865 that the pig was refractory to inoculation.

Luton in 1869 found, as Davaine had found, that dried charbonous blood retained its virulence.

Baillet observed a bacillus in the blood of all animals dead of "Mal de Montagne," which was recognized as charbonous by a Commission which was sent into the Cantal and the Puy de Dôme to study it. He supposed that the bacteridia or their germs, owing to the custom which prevailed of burying the animals in shallow graves in the pastures, lived on the soil and plants and were introduced with the forage.

In 1871, Klebs and Tiegel, having filtered charbonous blood through a clay filter, found that the plasma obtained without rods was not

virulent. This proved at least that the virulence did not reside in the liquid part.

Pasteur recognized the formation of spores in a pathogenic microbe connected with his studies on the causes of the diseases of silk-worms, and he found that these spores were very resistant bodies and were capable of germination several years after their formation; but this fact not having been verified in the case of the bacillus of Anthrax, showed Davaine's observations to be incomplete and allowed the discussion to continue.

P. Bert in 1876 discovered that oxygen at a pressure of several atmospheres killed anatomical elements and living beings, whilst it was inactive upon diastases, soluble ferments, and venoms; he submitted the bacillus of charbon to its action; he also submitted this virus to the action of absolute alcohol; and he observed in both cases that inoculation gave a rapidly fatal malady, without the appearance of the bacillus; in these cases the charbonous blood had become septic, contained spores, and produced septicæmia, the disease from which the inoculated animals died.

Koch in 1876 first observed the multiplication of the bacillus of charbon by the formation of spores; he cultivated it in blood plasma, aqueous humor, in the presence of air; he placed a minute quantity of charbonous blood in a drop of serum or aqueous humor; he saw the bacillus lengthen and form an inextricable felting of mycelium. He saw it not only form segments, but also very refractile spores, very close together and regularly spaced in the interior of the filaments. He saw the filaments become destroyed, leaving in their place spores laid out in a line and maintained in position by a uniting substance formed of the last vestiges of the filaments; he lastly saw the spores isolate themselves and become free. He inoculated the spores, causing charbon; he cultivated them in blood plasma, aqueous humor in the presence of air; he saw them stretch, lengthen, and form rods. He observed that they were more resistant and preserved their virulence for a longer period than the rods.

Briefly, Koch, by cultivating the bacilli in the aqueous humor or serum, in the presence of a moist atmosphere at a temperature of 35° to 37° C., saw the rods lengthen considerably, double, tenfold, one hundred fold, their length, turning about and forming an inextricable network or felting, lose in some hours their uniform structure and their transparency, their contents become granular; very shortly granulations or very refringent spores were formed more or less close together and at regular intervals, and resembling in their form and distribution interlaced chaplets of pearls. These dissociate and leave in their place lines of

spores which were maintained in position by a mucous uniting substance, but soon separated, becoming free. He observed that at 35° C. the bacilli rapidly developed and presented spores at the end of 24 hours, whilst at 30° C. they did not form them for three hours, at 18° to 20° C. at the end of two or three days. Below 12° and above 45° C. the bacilli did not develop. He recognized that the development of the bacilli required the presence of air, and that if cultivated in a medium poor in oxygen, they became muddy, broke up, and were destroyed.

Pasteur in 1877 devoted himself to the study of contagious disease; he completed the demonstration of the parasitic nature of charbon; he demonstrated, by employing the method of successive cultures inaugurated by him, that the bacillus was the morbific agent of charbon, and that, if the virus resisted the action of compressed oxygen, this was due to the great tenacity of the spores which it contained, and which, contrary to what is the case with the rods, are not influenced by it.

Koch had observed that the microbe of charbon might reproduce itself by scission or by the endogenous method. During the life of a sick animal, the batonnet multiplies by scission, elongates, is compressed, bends in one or several places, separates into several segments or batonnets, which in their turn comport themselves in a similar manner after death. The batonnets cease to multiply by scission, oxygen being wanting and septic bacteria get the upper hand. The bacilli, however, under certain conditions, produce spores before their destruction, which, once formed, may preserve and perpetuate the virulence for a long time.

Pasteur, as we have said, submitted charbonous virus to successive cultures outside the organism, and repeated the experiment a great number of times, and, at last, isolated the bacillus from all the elements which accompanied it in the drop of blood employed to sow the first culture; these elements did not multiply and finally disappeared; the bacillus alone multiplied and became purer in each successive cultivation; it was obtained pure under the form of filaments; and by inoculating the liquid which contained it, charbon was induced with multiplication of the bacillus. If the culture liquid or charbonous blood was filtered through porcelain, it was deprived of the bacilli and ceased to be virulent. The method of successive cultures, repeated a number of times, permitted us to get rid of all the elements which accompanied the bacillus, such as red and white globules, granular matter which were introduced in sowing the first culture, and transmitted in less and less numbers to some of the subsequent ones. The cause of charbon then is the bacillus and no other element, since the bacillus alone was preserved in the whole of the cultures, and neither a dissolved substance nor any poison whatever produced by it and

regenerating itself at the same time in the successive cultures, since once the bacteridium is removed from the blood or culture liquid, as is the case when it is filtered through plaster or porcelain, the malady cannot be produced by the inoculation of the liquid thus cleared. If it be supposed that the plaster filter causes any change in the plasma which traverses it so as to destroy in it any virulence which it might be endowed with, the contrary may be proved by using numerous double paper filters, or by leaving charbonous blood in a place free from germs. It separates into two parts; the plasma, obtained by decantation, is deprived of bacilli which have become caught and imprisoned in the coagulated part, and it does not produce charbon on inoculation. In addition to this the blood of the fœtus which does not contain bacilli is not virulent, which would not be the case were the virulence due to a liquid substance or poison. Toussaint, as Davaine had recognized before him, observed that the bacteria had all their virulence at the time of death before putrefaction had attacked the body, and that they were destroyed on the development of putrefaction; that they died, when they were deprived of air, if anything more rapidly at an elevated temperature. Having filtered charbonous blood through 4 to 8 thicknesses of paper, he found that the filter composed of four leaves allowed rods to pass and the liquid obtained cause a charbon when inoculated; whilst the filter of 8 thicknesses did not allow them to pass, and the liquid did not consequently induce charbon on inoculation. He found that it was possible to abridge the period of incubation by injecting a large number of batonnets into the blood. He cultivated charbonous blood in the aqueous humor and studied the transformations of the bacillus. It is then clearly demonstrated that charbon owes its appearance and development to the introduction into the organism of a microbe in the form of rods or spores. The bacillus is then the factor and the only factor of charbon, for in cultures we obtain it pure and free from any formed or *amorphous* particle.

Seat of the Virus—Characters of the Bacillus in the living animal and fresh cadavers.—The contagium of charbon is a microbe; and this microbe, as we have already seen, is met with in the blood of the sick, in their various organs, in their excretions, more particularly when the disease has arrived at its termination. It is notably abundant in the engorged spleen, in the diseased ganglia, in the vessels of congested organs, etc.; it may be found in the fæces, urine, in the discharge from the nose, and more particularly is this the case when the excretions are sanguinolent. Feser, Chambrelent, and Mossous, Koubassaf and Nocard have found it in the milk of the cow and of the guinea-pig.

The bacteridium of Davaine, still called Bacillus anthracis, presents itself in the blood of a sick animal, or shortly after death, under the form of batonnets or short rods *only*. These rods are either simple, or they may be formed of two, three, or more segments, straight, or sometimes crosier-shaped or bent and cylindrical. They are often articulated by an enlarged extremity presenting somewhat similar appearance to that of the articulatory surfaces of the phalanges. In the median line of these articulations is a clear, transverse line altogether characteristic. They vary in length according to the subjects from which they are obtained, and to their seat; they are longer in the guinea-pig than in the mouse, and in both than in the ox; longer in the exuded material, in the œdema which accompanies inoculation, than in the blood, where they are more rapidly divided into their individual segments on account of the movement to which they are constantly exposed; they also vary in thickness in different species and individuals; they are generally thinner in the ox than in the rabbit, etc. Their length may vary from 3 to 20 milimetres, and their breadth is between 1 and 1·5 m. The common appearance presented by charbonous blood in a fresh condition magnified 1,500 diameters is that the red corpuscles are agglutinated, deformed, and mixed with a profusion of small straight batonnets articulated or simple, but never presenting themselves under the form of long filaments. In the blood of guinea-pigs the bacilli are often, after 24 hours' inoculation, more numerous than the corpuscles which they smother, so to speak, by taking up the oxygen necessary for their life, and to that of the anatomical elements which receive from the blood the material for their nutrition. *The rods never contain spores when taken from a living animal or from a fresh cadaver.* They are composed of a very refractile protoplasm and of a less refractile envelop, which, however, is very resistant; around the rods may be observed a thin clear hyaline and gelatinous zone. There are as many unicellular microbes as there are segments: these are vegetable cells, always multiplying by segmentation or scission in the bodies of the sick. Segmentation is announced by the appearance of a transverse line, sometimes very thin, and which may only be evident on the application of reagents or heat. Seen in the blood, in the pulp of organs, the spleen, ganglia, etc., in the gelatinous exudation into the subcutaneous connective tissue, which contains less when less coloured and containing less hæmorrhagic infracts, they are easily recognized, even when examined unstained, on account of their elongated form, their refractility, immobility, numbers and arrangement, etc. In a drop of blood set up on a slide and examined with ordinary illumination, magnified 350 to 500 diameters, we ordinarily observe agglutinated red globules forming irregular masses and leaving spaces in which the rods may be clearly seen.

The bacillus resists the action of potash, ammonia, sulphuric acid, etc., preserving its form after having been submitted to the action of these substances. When we treat a ninfinitesimal portion of blood or dried pulp of an organ with a drop of acetic, or, better, formic acid, we see all the anatomical elements pale and disappear, whilst the bacilli persist perfectly clearly. The bacillus stains well with all aniline dyes, gentian violet, methyl blue and violet, fuchsine, rubine, etc. We may obtain beautiful preparations with the ordinary methods of a simple coloration, and more especially by double staining by Gram or Weigert's method. These preparations may be made with blood, exudate from diseased points, pulp of an organ, as the spleen, ganglia, liver, lung, marrow of bone, etc., epiploon villosities of the intestine, etc., and sections of organs previously hardened in alcohol.

Staining the rods.—Nothing is easier than to make stained preparations of the blood, fluids, and pulp of various organs. These take the form of cover glass preparations and are made as follows:—A small quantity of the material is placed between two cover glasses, which are then pressed gently and carefully together so as to expel any excess of the substance, leaving only a very thin layer between them; they are then gently drawn apart, sliding one over the other, and allowed to dry in the air, care being taken to exclude dust, etc. The thin layer which exists upon each cover glass is now fixed by passing it quickly several times over a flame, preferably that of a spirit lamp or a Bunsen burner, care being taken to keep the coated surface of the glass uppermost. They are now allowed to cool and are afterwards placed in a hydro-alcoholic solution of one of the aniline dyes for a few minutes; they are then washed with distilled water, then with spirit to dehydrate them, and cleared with clove oil or not, then mounted in Canada balsam and examined, preferably with an oil immersion and illuminated by means of an Abbe's condenser, when it is desired to make a minute examination. In simple staining the following method gives excellent results. Place the specimens in solution of Lœffler's blue, which is composed of one part of an alcoholic solution of methylene blue and three parts of a one in ten thousand solution of potassium, and allow them to remain in it for from a few minutes to half an hour; they are then rapidly passed through distilled water slightly acidulated with acetic acid and then treated as ordinary specimens.

Double-staining may be obtained by the method of Gram. The specimens are stained for five to ten minutes with Gram's solution composed as follows:—

Aniline water	10 parts
Absolute alcohol	1 part
Saturated solution of gentian violet	1 part;

they are then washed and placed for three minutes in the following :—

Iodine	1 part.
Iodide of potassium	2 parts.
Distilled water	300 parts.

They are again washed, decolorized by absolute alcohol ; washed again and stained with picrocarmine, or weak alcoholic solution of Eosin or Vesuvin ; they are finally washed, dried, and mounted. Double-staining may also be accomplished by Weigert's method, which is as follows :— Place for two to five minutes in a 1 per cent. watery solution of gentian violet, wash in alcohol, rinse in water, and transfer to picrocarmine solution (Weigert) for from half to an hour. Treat with alcohol until the colour is almost washed out.

Galtier recommends the following application of Weigert's method :— Commence by staining the preparation in picrocarmine for some minutes ; wash it in distilled water; then place it for fifteen minutes in Weigert's solution, made as follows :—

Cold saturated solution of methyl violet	6
Absolute alcohol	11
Aniline oil	3

wash and dry, then clear with a mixture of

White aniline oil	2 parts.
Xylol	1 part.

Wash in pure xylol so that no tint of picrocarmine remains, and then mount in balsam; the ground is rose-coloured and the microbes appear violet.

Preparations made with the epiploon or villi of the intestines may be coloured directly upon the slide or cover glass, in the same manner as preparations of the pulp of organs. We can place on the slide a small portion of the epiploon which contains only very fine vessels, or we may equally place a villus ; it is left to dry and is fixed by gently heating either in a stove or by passing through a flame, we then proceed as above directed. Sometimes with the epiploon we may adopt a different proceeding : we may stretch a piece across an excavated cork, pass it through a mixture of equal parts of alcohol and ether, then plunge it into Gram's violet ; afterwards treat with iodine solution, clear with absolute alcohol stain in eosine and mount like a section. Fine sections may be stained in the same manner.

Characters of the cultivated Ballcius.—The microbe of charbon may live, develop, and multiply outside the body, when the conditions necessary for its nutrition and respiration are combined with a temperature and degree of humidity suitable to it. It requires for its well-being a medium composed of nitrogenous and mineral material, such as serum,

aqueous humor, urine, vegetable infusions, broth, potatoes, gelatine, and peptonised jelly. It thrives well in neutral urine, but meat broths are preferable; it does well in gelatine or jellies as well as on potatoes. Air and free oxygen are necessary to its existence; it is "Ærobic;" it cannot be cultivated in vacuo, nor in the presence of carbonic acid, nitrogen, or hydrogen. It is not cultivated below 16° C. At 16° C. the bacillus does not form spores, at least for a very long time. It is cultivated rapidly at from 42° to 45° C., but at the latter temperature spore-formation is prevented or arrested. It does not grow in chicken broth at a temperature of 45° C. The temperature most favourable to the rapid development of the bacillus and the formation of spores is 30° to 35° C. The filaments are killed by a temperature of 60° C. or by carbonic acid or alcohol. The spores do not suffer any alteration from contact with the air, either in their vitality or their virulence; they resist the action of alcohol, carbonic acid, and vacuum, as well as, for a short time, a temperature of 95° centigrade; they are, however, killed with certainty at 100° C. When the conditions are favourable, the bacteridium multiplies at first by scission or segmentation as in the animal body.

The temperature of the blood of birds (41° to 42° C.) hinders multiplication, and in order to show that the temperature hindered development in the fowl, M. Pasteur cooled the bird by keeping the body in water, and so, on inoculation, produced the disease. It must be remembered, however, that in some exceptional cases birds may take charbon without being thus cooled down; for instance, the sparrow, which has a high temperature, is very susceptible to the virus. By the inverse process Gibier has been able to produce charbon in frogs and in fishes, by causing them to live in water at a temperature of 35° C. The blood of these animals, after inoculation, contained a large number of bacilli.

The culture media should be sown with pure charbonous material containing nothing but the bacilli, such as the blood, products of the spleen, ganglia, etc., taken with antiseptic precautions from fresh cadavers; and if we are only able to obtain an impure seed, it is necessary to sow it upon a solid medium, so that we may be able afterwards to separate the colonies of the different microbes.

Culture of the Bacillus in liquid media.—Broths made from beef, veal, chicken, etc., and of course sterilized and rendered slightly alkaline, are very suitable for the culture of the bacillus. We sow them with a drop of blood, or with the pulp of a charbonous organ, and place them in a stove or incubator at a constant temperature of 35° to 37° C. Under these conditions, the microbe of charbon rapidly forms in a few hours (about 24), numerous filaments, long, undulating, felting together, and forming snowy or cottony flocculi, easily seen in the culture liquid in

which they are held in suspension, and sometimes adhering to the sides of the tube; these flocculi enlarge and become more numerous; the culture liquid remains clear and limpid in the parts not occupied by the flocculi of filaments. The floccuent appearance, snowy or resembling cotton, is characteristic. By degrees the culture changes its appearance; after some days, the liquid becomes slightly brown or yellowish coloured, the flocculi disintegrate, and a white or slightly yellow powder forms at the bottom of the culture which spreads through the liquid and renders it turbid when shaken.

Culture of the bacillus on solid media.—Plate cultivations on nutrient gelatine may be made. The bacteria form colonies visible to the naked eye after one or two days, according to the temperature at which they are maintained. They appear to the naked eye as little white spots or specks which, when examined with a low power, say 60 diameters, with small diaphragm, exhibit two distinct forms. One form, on carefully focusing, has the appearance of a little compact ball of twisted thread; in the other, liquefaction of the gelatine has occurred, and the bundles are spreading out like locks of hair in the neighbouring gelatine. These appearances are perfectly characteristic.

Test-tube cultivation in nutrient gelatine.—Typically characteristic appearances are obtained by inoculating a 5 to 8 per cent. nutrient gelatine. Towards the second or third day the whole of the track of the puncture assumes a downy aspect, and a whitish coloured line develops, from which numerous fine filaments spread out in the gelatine. Occasionally a little isolated spot develops, from which rays extend in all directions, like the silky filaments of thistle down. These filaments are more easily observed with a magnifying glass. In a more solid nutrient gelatine the growth appears only as a thick white thread. As liquefaction of the gelatine progresses, these characters rapidly disappear, and the growth subsides as a white flocculent mass. In exhausted culture media, and sometimes in blood, filaments are seen in a state of degeneration.

Test-tube cultivations in nutrient Agar-Agar.—When a cultivation is made on a sloping surface of nutrient Agar-Agar, a viscous snow-white or creamy layer is formed.

Cultivation on potato.—A very characteristic growth results from the inoculation of sterilized potatoes. The damp chamber containing the spores is placed in the incubator, and in about 36 to 48 hours a creamy, very faintly yellowish layer forms over the inoculated surface, with usually a very translucent edge. On removing the cover of the damp chamber a strong penetrating odour of sour milk is encountered.

Cover-glass impressions.—The plate cultivation should be examined as soon as the colonies appear, by making cover-glass impressions and staining them, as should also the potato and other cultivations.

Characters of the cultivated bacillus.—Examined in the diverse stages of their evolution, the cultures show the bacillus under various forms. The filaments at the commencement contrast by their length with the relatively short rods, which is the form seen in the blood of the sick. They are very long, cylindrical, and larger than the rods in the blood, non-ramified, undulating, tangled into skeins or masses, and immobile. Examined in their natural condition in a drop of the culture liquid or in a piece of the culture on solid medium, after the addition of a drop of sterilized broth, they show the characters already noted; they appear homogeneous in their whole length, with rare segmentations, under these conditions; they exhibit a homogeneous protoplasm, transparent and devoid of granulation. Sometimes the filaments, when treated by reagents, show segmentation into short segments.

The filamentous bacilli are easily coloured by the various methods above indicated, by hydro-alcoholic solutions of gentian violet, methyl violet, fuchsine, rubine, methyl blue, methylene blue, etc., by Loeffler's blue, by the methods of Gram and Weigert. Staining allows us to observe the true structure and composition of the filaments. We then see that they are formed of a hyaline sheath, containing cubical or elongated protoplasmic masses, separated from each other by transverse partitions; each of these segments is a vegetable cellule, a microbe.

Examined after 24 hours, and day by day, the cultures show the bacillus under a new form—the spore form.

Spore formation consists in the appearance of a bright, glistening, spherical body in the protoplasm of an elementary mass or cell; these are the seeds of the plant, or, as we term them, spores. This body gradually enlarges until it reaches its full size, becoming at the same time oval. The bacilli are thicker at these points than where no spore formation has set in. Under the most favourable conditions, each cubical or rod-shaped mass of protoplasm includes one spore, in which case the bacillar filament contains an almost unbroken row of spores; but in other cases only an elementary mass here and there contains a spore, the rest breaking down and becoming absorbed. In the first case, also, the protoplasm of the elements almost entirely disappears, the sheath swelling up and becoming hyaline, and only the bright spores remain. Their linear arrangement, however, still indicates that they were formerly contained in one filament. After some days in an incubator, it is observed that a pulverulent deposit has formed at the bottom of the culture, and in it there are still some filaments or débris of filaments filled with re-

fractile spores; chains of spores still maintained in line by the jelly-like membrane of the filament; and lastly, masses of spores in a hyaline substance, which appears to support them, and free spores animated by brownian movements.

If bacilli grow in the depth of a fluid medium they do not form spores, and as new bacilli appear, or the old filaments increase in length, degeneration sets in. This degeneration gradually affects greater and greater numbers, and when the fluid is exhausted for the formation of new bacilli it necessarily follows that the whole growth becomes involved in degeneration, the whole mass becoming smaller, and finally only débris remaining. Such cultures—namely, those in which degeneration involves the whole mass of the bacilli—are quite innocuous when inoculated into animals or into fresh nourishing media. But as long as there are any good protoplasmic elements of the bacilli left, the culture remains virulent to rodents with the exception of mice, as will be seen later; and it is capable, when transferred to a new suitable nutrient medium, of starting new cultures which prove virulent to all rodents and sheep. The same holds good of the blood and organs of an animal dead of anthrax, provided the animal be not opened, and its organs, exudations, and urine be not exposed to free air; for the bacilli not exposed to the free air gradually degenerate, and the blood and organs of such an animal, although at first deadly poison to other susceptible animals, become at length quite innocuous. Systematic observations have shown me that small animals, such as mice and guinea-pigs, when unopened or buried in earth, become innocuous in from 5 to 8 days, the bacilli having by this time undergone degeneration and disappeared from the blood, spleen, and other organs. Pasteur's statement that in animals, dead of anthrax and buried, the bacilli form spores, and that these spores are taken up by earth-worms and carried to the surface of the soil, where they deposit them in their casts, is not borne out by these experiments (Cornil and Babes). Further, Koch has proved (Mattheil. a. d. r. Gesundheit samte, 1881), by direct experiment, that the spores of anthrax bacilli, when mixed with earth, are not taken up by these creatures.

Spores, as we have seen, form in the cultures; after the first days they form rapidly in cultures maintained at a temperature of 35° to 37° C. They also form at 30°, 25°, 20°, and 18° C., although more slowly; they become more numerous as the culture material gets more impoverished. Sometimes, under certain conditions, the cultures of the bacilli may lose the power of forming spores. Cultivated at 42° to 43° they do not form true spores (Pasteur); brilliant corpuscles, a kind of false spores, form in the filaments, smaller than true spores and not endowed with like powers of resisting heat (Chauveau). But these

bacilli without spores, sown in broth and cultivated at a temperature of 35° to 37° C., produce spores; they are, therefore, not rendered truly asporagenous by cultivation at 42° to 43° C. At too low a temperature the bacillus does not form spores, but it does not lose the power of doing so when cultivated at a proper temperature; spore formation does not take place below 15° C. In 1883 MM. Chamberland and Roux obtained asporagenous bacteridia, by causing weak antiseptics to act upon the cultures, using phenic acid and bichromate of potassium for the purpose. In this manner the bacillus cultivated for eight days in a culture medium to which a two-thousandth of bichromate of potash was added, lost the faculty of producing spores, and remained asporagenous in successive cultivations; it killed guinea-pigs in three or four days, and the cultures sown with the blood of the charbonous guinea-pig remained asporagenous. Lehmann, in 1887, discovered another method of producing asporagenous bacilli, which only formed false spores, and which did not regain the power of forming spores after having been passed through guinea-pigs and mice, and his method was repeated cultivation on gelatine. Behring, in 1889, obtained similar bacilli by cultivation on gelatine, to which rosalic acid or an antiseptic had been added in a dose insufficient to kill the microbe. M. E. Roux has pointed out a certain method, and one easy of application, of obtaining cultures of asporagenous bacilli. The following is a *résumé* of his method:—Take eleven test tubes and place in them ten cubic centimetres of veal broth rendered slightly alkaline; leave one as a control, and add to the others weak doses of phenic acid, such as 2 ten-thousandths to one, 4 ten-thousandths to a second, and so on, in such a manner that from the first to the tenth there is a progression of 2 ten-thousandths per tube; sterilize the tubes at 115° C., after sealing those containing the acid in order to avoid loss; then sow each of the tubes with a drop of blood, and put them in an incubator at a temperature of 30° to 33° C. The bacilli develop, if anything, more slowly as the dose of phenic acid has been larger. The culture is always less abundant in phenicated broth than in the ordinary broth, and it generally occurs in flocculi, which remain in the depth of the liquid, if the tubes are not shaken; at other times the development occurs in the whole mass of the liquid, and gives it a troubled appearance; it is more especially in the tubes, which are richer in the antiseptic, that this appearance is produced. We should, if possible, avoid the cultivation occurring at the surface of the liquid and forming a crown to it, which adheres to the side of the tube. The bacilli, which increase in this manner, freely exposed to the air, outside the culture liquid, quickly form spores, principally in those tubes poor in phenic acid. When, therefore, flocculi appear, we submerge them by agitating the tubes a little. The

broth, to which 20 ten-thousandths of phenic acid has been added, remains sterile, the bacilli sown in it rapidly perishing.

After eight or ten days we take a little of the culture from each of the tubes and heat it for fifteen minutes to 65° C., and then sow the heated portion in ordinary veal broth; in a couple of days the sowings made with material from the tube to which 2 ten-thousandths of phenic acid have been added show themselves to be fertile. Those made with material from the tube containing 4 ten-thousandths and 6 ten-thousandths often give a culture on the following days, but the others remain for the most part sterile, and so prove that the bacillus sown was without spores.

The proportion of the antiseptic necessary to prevent the formation of spores varies with the composition of the broth, the origin of the bacilli, and the facility with which air gains admission to the culture. It is for this reason that we take the precaution of using a number of tubes with slightly varying quantities of the antiseptic added to them, and differing in no other respect; unforeseen results often occur in these experiments. We may find, for instance, a tube containing 6 ten-thousandths of the antiseptic produce no spores, whilst one sown in 10 ten-thousandths may contain them, although the two tubes have been sown with the same blood. The bacillus does not suddenly lose the power of forming spores; if we remove it from the antiseptic medium, after three or four days of culture only, it will form spores if we sow it in a non-antiseptic broth. In order that it may become asporagenous it is necessary that the contact with the antiseptic be prolonged.

Sown in ordinary broth, without the application of heat, the asporagenous phenic cultures rapidly develop at 33° C.; and the daughter cultures have no spores; even after a long time they perish without having formed them. We may make successive cultivations of these bacteridia, either in broth, gelatine, nutritive gelatine, coagulated serum, or liquid serum, without their forming spores. They have not, however, lost their virulence; inoculated into guinea-pigs they kill them in from 30 to 36 hours and rabbits in from 48 to 60 hours. At the seat of inoculation there is extensive œdema in guinea-pigs, generally greater than what occurs after the injection of the ordinary bacillus. When we pass this asporagenous bacillus through a large number of guinea-pigs and rabbits, inoculating a healthy animal with blood from a dead charbonous animal, it does not regain its power of producing spores in cultures, even though its virulence increases. Blood of the rabbit dead of asporagenous charbon may be placed upon the side of a sterilized tube closed by a plug of cotton wool, and left for fifteen days without producing spores in a moist chamber, in contact with the air, and at a temperature of 30° C. We know that ordinary charbonous blood, placed

under these circumstances, rapidly produces spores. The aqueous humor of a rabbit's and the sheep's eye is a particularly favourable medium for the production of spores, and the ordinary bacillus gives, in it, after twelve hours, beautiful spores, capable of resisting a temperature of 15° C. and 190° C. The asporagenous bacillus does not produce spores even when cultivated in fresh, aerated aqueous humor. This, then, is no temporary and passing change of the bacilli of charbon, but a permanent hereditary change, and at present we are not acquainted with any method by which we may endow the bacilli so modified with the power of producing spores.

The appearance of cultures of asporagenous bacilli closely resembles that of ordinary cultures of the bacillus. In broth they give flocculi which are more easily broken down; the filaments of the asporagenous bacillus are shorter and slightly thinner; they often contain in their interior refractile grains, smaller than spores, and not resistant to heat. At the commencement of the culture the filaments are transparent and stain well; in proportion as the culture ages, many of them become granular, swell up and stain badly. Cultivated on gelatine, the asporagenous bacilli appear to liquefy it more rapidly than do the ordinary bacilli. Broth in which the sporeless bacilli develop is less coloured in the incubator, and gives less abundant crystals of ammonio-magnesium phosphate than that which has nourished the ordinary bacillus of charbon. The cultures of asporagenous bacilli finally perish at 33° C. after a longer or shorter time, but generally within a month. If after some days in the incubator we place them at the temperature of the air, they remain alive for a much longer period. Sporeless cultivations have been preserved in this manner sown in broth for 155 days, at the end of which time they have given cultures; they did not, however, contain any spores, and after heating at a temperature of 65° C. for fifteen minutes were sterilized. In other cases, these asporagenous cultures remained in a cupboard in the laboratory, being still alive after 81 days; a culture on nutrient jelly was still alive after 47 days. In these old cultures very few bacilli remained alive; to resuscitate them they should be sown in large quantity, and after several days we may perceive development taking place; the virulence of these cultures without spores persists until they perish. A culture taken a short time before its death or the death of its filaments is active and destroys guinea-pigs and rabbits. In the mother cultures, on the contrary, made in the presence of an antiseptic, there is a diminution of virulence, as MM. Chamberland and Roux have long since pointed out. The daughter cultures made from phenic cultures about eight days old—that is, at the time when the loss of virulence is least marked—do not weaken sensibly in successive cultures in broth. However, it seems that these

sporeless bacilli must be in a condition particularly favourable to attenuation, since they remain exposed in the form of filaments to the atmosphere at the temperature of the incubator. It is to the action of air upon the mycelium without spores that MM. Pasteur, Chamberland, and Roux have attributed the attenuation of the virulence of the cultivated bacilli at a temperature of 42·5° C. These two conditions—the absence of spores and the continued action of the air on the filaments—are realized in the case of our asporagenous cultures, but in spite of this they do not become attenuated. We must consequently come to the conclusion that the action of air at a temperature of 33° C. is not sufficient to produce a diminution of virulence; it must be increased to a higher temperature. It would be interesting to know at what temperature it is necessary to make the culture in order to bring about attenuation of the virulence of asporagenous bacilli. We may also obtain attenuated asporagenous bacilli either by sowing, as M. Behring has done, bacilli already attenuated in a medium containing a small dose of antiseptic material or by prolonging the contact of virulent bacilli with phenic acid, as we have indicated below. To obtain virulent asporagenous bacilli we may sow charbonous blood in a phenicated broth, and after eight or ten days' culture remove a small quantity of seed and place in sterilized tubes. The bacilli have remained for too short a time with the antiseptic for their virulence to have become profoundly altered, and the daughter cultures so obtained are sporeless and still kill guniea-pigs and rabbits. If we leave the bacilli for a sufficiently long time in the presence of the antiseptic, its virulence will diminish, as is well known, and will be incapable of forming spores or of killing animals.

These asporagenous bacilli give a novel and very interesting example of the permanent and hereditary modifications which microbes may be caused to undergo. Since the observations made in M. Pasteur's laboratory we know that virulence is a quality which microbes may lose and acquire, and that pathogenic properties are not sufficient to characterize species. The morphological characters are no more certain in many cases. The manner in which the bacillus forms spores, the resistance of these to various agents, and the fashion in which they germinate in suitable media, have been regarded as very characteristic particulars. It is, however, seen that the power of forming spores may be modified to such an extent as to cause its permanent loss. The action of antiseptics is probably not the only method by which this modification may be brought about. Lehmann had not added any antiseptic to the cultures in which he found bacilli without spores; he did not know to what influence to attribute this modification, which was produced without any intervention on his part. It is then not beyond

possibility that in nature conditions may be realized which transform ordinary bacilli into attenuated and asporagenous ones. The experimentalist, who should meet a similar bacillus, would be very much puzzled to recognize in it the "Bacillus of charbon." He would not have the slightest hesitation in regarding it as a species of Saprophyte without associating it with the bacillus anthracis. It is necessary, in fact, to have followed step by step, in well-conducted experiments, all the series of changes of the bacillus to be certain that the inoffensive and sporeless microbe is the virus of charbon; microbes are easier to modify than is generally admitted. It is with great trouble, in fact, that in our laboratories we preserve them with their characteristic appearance, in careful conditions of culture. We are not justified either in declaring that two microbes are not related to each other because their colonies are not quite the same, and one of them liquefies gelatine more rapidly than the other. What are these differences when compared with that which exists between attenuated sporeless and virulent spore-bearing charbonous virus? Insignificant in truth, and they should not authorize any experimentalist to regard as issues of different stock, two microscopic organisms very similar in other respects. The dissimilarity between microbes of the same origin is due doubtless to the diverse vicissitudes to which they have been exposed, and to incidents with the nature of which we are unacquainted. It is sufficient for us to avoid attaching too much importance to artificial characters and distinctions.

If the work of attenuation of the virus prove to us that virulent and fatal microbes may become inoffensive, the converse, or the return to virulence of these attenuated microbes, leads us to suspect that a saprophyte may become virulent. The pathogenic organisms with which we are at the present day acquainted may be ancient saprophytes which have become progressively adapted to parasitic life. These ideas, enunciated often enough before, explain the interest which biologists and pathologists attach to experiments which furnish well-established examples of these permanent modifications of microbes.

In old cultures we may find abnormal forms, filaments with swellings, irregular or ovoid, or with a series of granulations varying with their size. The spores of the bacillus are very refractile, and it is easy to distinguish them from pseudo-spores, either by staining, or, better, by heating them for fifteen minutes at a temperature of 65° C., which destroys the filaments and all material not true spores without destroying spores which resist a temperature of 90° C. for fifteen minutes. The spores are difficult to stain, not being affected by ordinary dyes, and herein they differ from the bacilli. We may, however, stain them in several ways; firstly, by heating them for nearly an hour at a temperature of 200° C., or by passing the slide or cover glass, upon which is

placed and dried the drop of the culture, through flame, then treating them with a hydro-alcoholic solution of gentian violet or some other stain. Secondly, by leaving them for 24 hours in a warm solution of fuchsine in aniline water (Erlich's solution), decolorizing with nitric acid 30 per cent. or by absolute alcohol, and exposing them to the action of a hydro-alcoholic solution of methyline blue. By the first of these methods the spores alone are stained, the elevated temperature having so altered the filaments as to render them unable to fix the coloring matter and the spore able to do so. In the second method a double coloration is achieved—the spores are stained red and the filaments blue. In conclusion, then, we may repeat that the bacillus of charbon, cultivated in suitable media, such as broth, produces, after from 24 to 48 hours, excessively long filaments disposed in such a manner as to form flocculi resembling cotton wool; after the lapse of a further period the filaments become filled with refractile corpuscles, then lose their contour, and being destroyed, leave in their place chaplets or spores, which later on become free, grouped into masses, preserving their lines, and capable of growing into batonnets or rods or filaments when they are inoculated or sown afresh. Spore formation occurs when the conditions of the life of the organism become least favourable, when the medium in which cultivation is going on becomes poor in nutritive material or oxygen, when the temperature is low, etc., etc.

The blood of a charbonous animal, or the excretions containing it, must be exposed to the air in order that spores may be formed. It is perfectly easy to follow the evolution of the culture and the modifications of the microbe under the microscope, by sowing it in serum or aqueous humor placed in a moist chamber and kept at a temperature of 33° to 35° C. by means of a warm stage. We see the rods double their length at the end of an hour, elongate to ten times their original length in two hours, and become transformed into long filaments in seven or eight hours; then we see small, very refractile, rounded or ovoid masses appear in the filaments; these are the spores, which are composed of a very refractile protoplasm and a very resistant envelope. The spores of charbon form rods or filaments only with difficulty or not at all in the liquid where they have been produced. However, few of the cultures are free from filaments, and it is not impossible that the spores may sometimes germinate in the liquid in which they have been formed. Be that as it may, the spores inoculated or sown in suitable media produce *rods in the organism* or *filaments in the culture media.* The cultures of the bacillus produce the disease in animals which receive them, just as do the charbonous products or blood of the sick.

Antagonism between the "Bacillus" of Charbon and other Microbes.—The microbes of charbon disappear in the presence of those

of putrefaction when they are sown in the same medium. Their development may be retarded or stopped in culture media when, either intentionally or unintentionally, they are sown with other ærobic microbes as some common bacteria are; in such cases it may happen that the microbe of charbon develops little or not at all, or that it perishes after a certain time. Inoculated alone into the organism they generally kill, although they have to battle against the cells; but inoculated in company with common bacteria they may perish without causing any disturbance. (Pasteur.) The antagonism is not always so marked; and the microbe of charbon may develop at the same time as others in the same medium; as, for instance, in the milk of the cow attacked with specific mammitis sown with the cultivated microbes of the two diseases. (Nocard.) Nevertheless, the antagonism of the microbe to other microbes is worthy of mention. We know that the bacillus anthracis develops badly in a medium wherein the microbe of fowl cholera already exists, that it is possible to render inoffensive the inoculation of the microbe in that rabbit by preceding it with, accompanying it by, or following it by the inoculation of the microbe of erysipelas (Emmerich, Watson, Cheyne, Pawlowsky); that the inoculation of certain inoffensive or pathogenic microbes (bacillus prodigiosus, staphylococcus pyogenes aureus, pneumococcus) may also exercise a certain protective influence against the microbe (Pawlowsky); that the microbe of "Rouget" may hinder the effects of the microbe of charbon on the guinea-pig (Zagari); that a saprophyte bacterium closely resembling the bacillus anthracis, growing and giving spores like it, but without its pathogenic action on small animals, and capable of giving them a certain immunity against the bacillus anthracis, has been found in air and water (Hueppe and Wood).

Again, new observations have been made in these later days relative to the antagonism of the bacillus to other microbes. Bouchard, Frendenrich, Guignard and Charrin have remarked that the pyocyanic bacillus lessens the infection of anthrax in the rabbit and guinea-pig; it has also been found that sterilized pyocyanic cultures exercise an antagonistic action to the bacillus of charbon (Woodhead and Cartwright Wood).

Boin and Rœser have found that yeast, which is not harmful to the organism, lessens the effects of the virus of charbon; and Buchner has observed that sterilized cultures of the bacillus of Friedlander may arrest the development of charbon.

By what mechanism is this effect of one microbe upon another produced? is it the direct influence of one microbe or its products against the other, or is the system not excited to intervene more energetically? The reply to this question is in part decided by the researches made by

Blagavettchensky. This investigator has found (1) that the simultaneous inoculation into the eye of pyocyanic bacilli and the bacillus of charbon generally opposes the development of the latter without, at all events in the immense majority of cases, rendering the animal refractory to a future charbonous infection; (2) that pyocyanic bacilli inoculated at some little distance from charbon bacilli exercise a much slighter effect upon them; (3) that the pyocyanic bacilli exercise a direct action upon the bacilli, hindering their development; (4) that successive injections of the two microbes into a being do not prevent the development of charbon; (5) that sterilized cultures of the pyocyanic bacilli can only influence the development of the bacilli when used in very large doses; (6) that outside the body development is arrested; (7) that the proximity of the products furnished by the pyocyanic bacillus exercises a harmful influence on the development of the bacteridia; (8) that the products of the bacillus exercise the harmful influence.

Resistance of the "bacillus" to Physical and Chemical Agents.—The microbe of charbon, as we have seen, presents itself under two principal forms—under the form of rods or filaments and under the form of spores. Its resistance to the various agents, which have the effect of sterilization, is very variable according as it is found under one or the other of these forms. The rods preserve their virulence and their lives for a short time only, when they are deprived of air or oxygen; they are destroyed without forming spores and are resolved into inoffensive granulations when they are placed in a situation deprived of air or oxygen, or in an atmosphere of carbonic acid gas; they are in this case asphyxiated more quickly when the temperature is elevated to near 38° to 40° C. Charbonous blood taken with due antiseptic precautions from a living animal or from one recently dead, does not contain putrefactive microbes; it only contains charbonous rods; it loses its virulence after a certain time, which varies according to the surrounding temperature, when placed in a vessel containing no air or in an atmosphere of carbonic acid; to vegetate it requires the presence of air.

We have seen that the microbe only vegetates at certain temperatures—from 12° to 43° or 44° C.,—and that it does only form spores between 15° and 43° C.; above 45° multiplication by segmentation ceases. Low temperatures have no great influence on the microbe; the rods resist 45° C., and the spores are not killed by a temperature of 70° C. or even 150° C. Rods and filaments are killed by a temperature of 100° C., by cooking the media in which they are found, and even by a temperature of 60° C. or even 55°, 50°, and 45° C., and they are exposed for a sufficient length of time. The spores have a greater power of resistance and bear heat much better; they resist a temperature of 95° for ten minutes in a

moist atmosphere, and can bear 80° C. for some time ; they are capable of resisting temperatures of even 100°, 110°, 115°, 120° and 123°, when they are ancient, dried, or heated in dry air (Koch). Sometimes, however, contrary to the observations of Koch and Wolffhugel, who have seen spores not destroyed by a heat of 140° until after three hours' exposure, M. Massol has found that they are always destroyed after an exposure of 57 minutes in dry air at a temperature of 100° C.

To resume, spores resist a temperature of 95° C. for ten minutes in a moist atmosphere, whilst they are destroyed in less than five minutes when the temperature is carried to 100° C. Below 80° C. they preserve their vitality for a considerable time; we may heat them for a number of hours at 70° without destroying them (Roux). All the spores of a culture do not present the same degree of resistance to heat.

At a temperature of 70° C. the spore of charbon does not perish for a very considerable time when heated without the presence of air; in the liquid in which it was formed this long heating does not sensibly modify its virulence; it is necessary that the heating be prolonged to nearly the limit of the resistance of the spores for the virulence to be lessened ; and, on the other hand, even though by this means the virulence is lessened, its lessening does not appear to be of the nature of a true attenuation, for bacilli born of spores which have been heated in this manner for 165 hours at a temperature of 70° C., kill guinea-pigs and rabbits. At the same temperature the spores, heated in the presence of the air, die more quickly; and, before dying, lose their virulence, or it becomes sensibly diminished. Inoculated into animals they remain inactive from 24 to 36 hours, but their culture is still virulent. Sometimes, when we inoculate cultures of spores heated in the air for a longer period (64 hours), a real attenuation of their virulence may be observed which may be preserved in successive cultures. Oxygen, on account of its oxidysing properties, is then the agent which interferes most with it. Spores already attenuated by Pasteur's method are more easily acted upon by heat than are spores which have not been attenuated. Spores heated in the presence of oxygen undergo the same modifications, with little less rapidity than those heated in the presence of air.

The filaments from spores heated in air or without air rapidl y form spores in cultures (Roux).

Rapid desiccation kills the rods and filaments ; slow desiccation does not prevent the formation of spores, which explains how it was that Davaine found charbonous blood virulent a long time after desiccation. The spores once formed bear desiccation, preserve their properties for a very long time, resist putrefaction, alternate lowering and raising of temperature, humidity, desiccation, etc. Dried spores may preserve their virulence for years.

The action of light upon the microbe is important. M. Arloing's experiments led to the following results: 1stly, that gaslight modifies the vegetation of the microbe but little, that it retards the evolution of the spores sown in broth, without modifying their pathogenic properties; 2ndly, that sunlight is more active, hinders the evolution of the spores and the development of the bacillus, and gradually attenuates cultures; 3rdly, that exposure to the sun for two or three hours in July, between 35° and 39° C., destroys the power of vegetating in broth culture media freshly sown with spores; 4thly, that it requires 27 to 30 hours' exposure to the sun, in the same conditions, to sterilize the mycelium of the same bacillus in a state of full development, and that before death takes place the microbe undergoes a series of progressive alterations which transform it into a vaccine of less and less activity.

It had been considered that the least resistance of the spores, shown by these results, was due to the fact that those which were sown commenced to germinate despite the action of the rays of the sun, and that the young mycelium in course of formation rapidly undergoes the sterilizing influence of the sun's rays. And this explanation of the phenomenon appeared to be demonstrated by the results obtained by M. Straus, who observed, with regard to the resistance of the spores, that when they are immersed in pure water germination does not occur for four hours, whilst in broth it may commence rapidly (in two hours). Nevertheless, M. Arloing obtained death of the spores after an hour's exposure to the rays of the electric light acting on cultures kept in ice at a temperature at which germination is impossible. In addition to this, putting aside the intimate mechanism which presides over sterilization, it remains an established fact that exposure to light more or less rapidly destroys the spore of charbon, that it may kill it very rapidly, or not till 29 or 54 hours have elapsed (Roux), and that its action is more or less rapid in proportion to the intensity of the light and according to the surrounding medium which, being modified, when culture has already commenced, renders the sterilization slower. On the other hand, it is averred that the action of light is due to the presence of oxygen, and that the spores of bacillus anthracis live longer when they are exposed to the action of light without air. Similarly, M. Roux, experimenting on the spores of charbon, believed that the action of light is due to that of oxygen, and that the action of light favoured the action of the air, and that in the absence of air the spores are modified much more slowly. Under the combined action of light and air there is produced in a culture medium a chemical change which arrests the evolution of the germs; generally, flasks containing broth and the germs do not cultivate when placed in a stove, after two hours' exposure to light; whilst those

containing only broth after two hours of exposure to the action of light, and to which spores are afterwards added, cultivate for the most part when placed in the incubator; nevertheless, when the broth has been exposed for three or four hours the spores shown in it do not germinate. Yet the spores are not killed neither after two hours' nor after seven hours' exposure to the action of light; and when shaken in a medium which has been exposed to the action of light, and in which they will not germinate, then sown in a medium which has not been so exposed, they vegetate; germination is, it is true, if anything, the more retarded the longer the spores have been exposed, or the longer they have been kept in the medium which has been exposed to the action of rays of light. Exposed broth, in which the spore does not develop, is suitable for the cultivation of the filaments; germination is arrested by a modification of the medium, which is insufficient to arrest the multiplication of the rods. Oxygen plays an important part in the modification of the medium, for, after exposure, the spores exposed in a medium deprived of air germinate in the same medium when it is aerated, whilst those which have been exposed to the action of both light and air do not germinate when so treated. In addition to this, broth deprived of air or placed in an atmosphere of carbonic acid may remain exposed to the action of light for many hours and afterwards may serve for the cultivation of the rods or spores; whilst a few hours' exposure to light and air renders a broth unsuitable for the development of the spores. The action of light and air is rapid when the receiver is freely open and contains only a small quantity of broth. We may increase the effect of light and air by adding to the broth substances which oxydise easily; in such manner, veal broth to which glucose has been added, becomes, after exposure, more unfavourable to the germination of spores than broth to which sugar has not been added. A medium which has become unsuitable to germination after the exposure may regain its original properties when kept in diffused light or partial darkness. Cultures of spores which have been exposed to the action of light without air, preserve their germinating power for a long time, becoming slower and slower in performing the process as they remain for a longer period in the medium. Cultures of spores which have been exposed, without air, for 93 hours, have not sensibly lost their virulence. It is the same with cultures of spores submitted for 54 hours to the action of light and air. The filaments, as M. Arloing has demonstrated, exposed to the sun and air in broth for a sufficient length of time, become by degrees attenuated, and their cultures follow the same course (Roux).

Under the double influence of oxygen and light, oxydation, which is hurtful to the life of the microbe, occurs. It is averred lastly by

Duclaux that spores exposed in a dry state may preserve their power of vegetating for a month or more. The oxygen, which is necessary for the development of the bacillus anthracis, may, under such circumstances, contribute to its attenuation, or may even destroy it. Its action is more particularly marked in adult bacilli, the spores bearing its presence much better. Compressed oxygen also acts more energetically on the rods or filaments than on the spores. When chemical germicides are caused to act upon the microbes we still observe great differences between the resistance of the rod and that of the spore; the adult bacilli are killed more rapidly and with much smaller doses than are the spores. Bromine, iodine, and chlorine may be used as germicides when we are dealing with the bacillus anthracis even in the spore form. According to Davaine 7 milligrammes of iodine sterilizes a litre of fluid in which has been placed 1 centimetre cube of charbonous blood; it arrests the development of charbonous microbes in culture media in which it has been dissolved, and kills the spores after 24 hours' contact (Koch). Charbonous spores perish after an hour's exposure to chlorine water and the rods are destroyed more quickly (Perroncito). Chlorine gas, 25 to 50 per cent., kills the spores of charbon in a minute (Galtier), but in practice these agents are little used. As to sulphurous acid, we know that it is unable to sterilize the spores of charbon; and after the experiments of M. Thionot we cannot count on sulphurous acid in large quantities used for 24 hours to destroy the microbe of charbon. Essence of turpentine which has been considered as capable of destroying the bacillus and its spores (Pasteur), may be inactive against the latter (Koch). Solution of nitric acid must be used in high percentage to kill the spores, whilst a 2 per cent. solution of hydrochloric acid should be sufficient to kill the bacilli and their spores (Koch). A 5 per cent. solution of cresyl rapidly destroys the adult bacillus; but the spores resist its action for a considerable time—24 hours according to some, and more than 20 days according to others (Esmarch). With regard to the action of carbolic acid the following remarks by Koch may be studied with benefit:—"Anthrax spores, dried upon silk threads, were placed in carbolic solutions of various strengths and kept in corked bottles. A thread was removed from time to time, and planted on nutrient jelly. The following shows the results:—

Carbolic Solution.	Days of Exposure.
2 per cent.	1, 3, 5*
5 „	1, 3
1 „	1, 2, 3, 4, 5, 7, 15
2 „	1, 2, 3*, 4*, 5*, 7*
3 „	1, 2*, 3*, 4*, 5*, 7
4 „	1*, 2*, 3, 4
5 „	1*, 2, 3, 4

"The plain figures in the second column signify that the spores removed from the disinfectant solution on those days developed on the nutrient jelly as actively as in the control experiments, and had therefore not been affected by the action of the carbolic solution. The figures bearing an asterisk signify that the spores transferred to the jelly on those days yielded scanty or retarded growths, less active than those of the control experiments. The doubly underlined figures indicate complete sterilization—that is, that the spores removed from the carbolic solution on the corresponding days yielded no growth whatever upon the jelly.

"Thus 1 and 2 per cent. failed to destroy anthrax spores within a week; 3 per cent. took seven days; 4 per cent took three days; and 5 per cent. required more than one day.

"The action of carbolic solutions upon the bacilli destitute of spores were of a very different character. Bacilli from the spleen of a mouse, dead of anthrax, in which spores were not formed, were dried upon silk threads and subjected to the same experiment. Two minutes' exposure to a one per cent. solution killed the bacilli. It was also found that blood from an animal dead of anthrax, mixed with an equal volume of 1 per cent. carbolic solution and allowed to stand for a short time, did not infect an animal into which it was injected; but if the strength of the carbolic solution were only 0·5 per cent., anthrax ensued.

"With regard to the inhibition of the growth of bacillus anthracis, threads charged with anthrax spores were placed in a series of seven vessels, each containing 10 cc. of fresh clear blood serum; and to these were added respectively 1, 2, 4, 6, 8, 10, and 15 drops of a 2 per cent. carbolic solution. They were protected from evaporation and pollution by dust by means of a cover. In the first four the growth was as active as in the control experiments; in the fifth it was delayed, but ultimately equally vigorous; in the sixth there was only a scanty growth of short and crooked threads; in the seventh there was no growth whatever, but the spores still grew vigorously, after 72 hours' immersion, when transferred to fresh nutrient jelly.

"These experiments were repeated with similar quantities of fluid, the blood serum, however, being replaced by a solution containing 1 per cent. of peptone, and ½ per cent. of meat extract. Five drops of 2 per cent. carbolic solution had no effect, ten drops caused marked retardation, and twenty drops entirely prevented the growth.

"Hence one part of pure carbolic acid in 850 parts of the cultivating fluid completely prevents the growth of anthrax bacilli; whilst one part in 1,250 causes marked diminution of activity."

Perroncito states "that 2, 3, 4, 5 per cent. of solution of carbolic

acid rapidly destroys the rods and filaments of bacillus anthracis, but the spores resist a 5 per cent. solution for 24 days."

Koch also remarks of several compounds of carbolic acid which were tested as to their power of destroying anthrax spores, but all proved to be less powerful than the pure acid, and he decided that carbolate of zinc stands next to carbolic acid as a disinfectant.

Anthrax spores were found to be absolutely unaffected by lying for 110 days in a 5 per cent. solution of carbolic acid in oil, or 70 days in a solution in alcohol.

Even the sensitive anthrax bacilli were not more affected by 5 per cent. solution of caroblic acid in oil than by pure oil, as will be seen from the following table:—

	Days of Exposure.
Carbolic acid, 5 per cent. in olive oil . .	1, 2, 3*, 4*, 6 =
„ „ 1 „ „ . .	1, 2, 3*, 4*, 6 =
Pure olive oil	1, 2, 3, 4*, 6 =

The same holds good as regards thymol and salicylic acid. It might be supposed that the enveloping membrane of the spores is impervious to carbolic acid unless softened by the action of water upon it, but the action of carbolic and other vapours on the dry spores tells against this view. If carbolic oil comes into contact with substances containing water,—the tissues of the body, for instance,—doubtless part of it is taken up and exerts some antiseptic action. It appears that sulphuric acid must be used pretty strong to destroy the spores of charbon; absolute alcohol kills the adult rod, but not the spore. Weak solutions of potash and soda destroy the rods, whilst the spores resist solutions of from 7 to 20 per cent. (Perroncito). A 5 per cent. solution of permanganate of potassium sterilizes the spores of charbon. Chloride of lime in strong solution instantly destroys the adult bacillus, and can kill the spore in five minutes (Franz Nissen). Lastly, bichloride of mercury is with hydrochloric acid and chloride of lime an agent to be recommended. The solution at 1 per mille, and more particularly stronger solutions varying between 2 and 5 per mille, may be advantageously used in practice even against the spores. Davaine observed that a solution of one in a hundred and fifty thousand could destroy the virulence of charbonous blood. Koch found that even in the enormous dilution of 3 parts per million, it entirely arrests the development of the bacilli. Further experiments showed that 1 per 1,000 watery solution of mercuric chloride, mercuric nitrate, or mercuric sulphate destroyed anthrax spores in ten minutes. Anthrax spores, which had been exposed for ten minutes to a 1 in 20,000 solution of mercuric chloride, and then washed in alcohol, gave no growth

upon gelatine, whereas, after exposure for an hour to a 1 in 50,000 solution, the growth was as vigorous as in the control experiment. Quinine impedes the development of anthrax bacilli at a dilution of a 1,200 per million, and entirely prevents it at 1,600 per million.

What becomes of the microbe in the cadavers, cadaveric debris, water and soil.—In the bodies of animals dead of anthrax, putrefaction, as we have already seen, is promptly established, because the vibriones of septicæmia which always exist in the digestive and the air passages, where they are introduced by food, water and air, not being impeded by the action of the cellular elements, which have lost their vitality too greatly, find themselves in a particularly suitable medium, more especially as the bacilli of anthrax have left little oxygen; they consequently rapidly develop and invade all the organs. For this reason, when we wish to examine the blood of an animal to decide whether or not he has succumbed to anthrax, it is necessary to do so as soon after death as possible, and it is also imperative to take antiseptically, as soon after death as possible, products which we intend to examine later on. A few hours after death the action of the septic vibriones may mask the result, and the inoculation of a charbonous material which has become septic lead to septicæmia.

In the blood and organs of animals dead of charbon, the bacilli require air for their existence which is not forthcoming, they find themselves in the presence of the carbonic acid given off by the septic vibrione, and the temperature not being a suitable one, as a rule, they do not form spores nor filaments, although they may ocrasionally elongate in the blood, and they do not consequently develop; they perish more or less quickly.

So, then, if they do not form spores in the blood of the sick animal, as we know they do not, it is the same in the cadaver, so long as the blood does not become exposed to the air; for if after two or three days we remove blood from a cadaver and heat it to 60° or 70°, a temperature sufficient to destroy the rods but not the spores, it does not fertilize a culture sown with it. In the same manner charbonous cadavers lose their virulence if anything more surely and more quickly according as putrefaction comes on quickly or slowly. Everything which facilitates putrefaction hastens the destruction of the microbe. The length of time during which the bacillus anthracis remains alive after the animal's death varies with the temperature; cold—a temperature below 10° C.—facilitates its preservation. We may find it living after four or five days in a moderate climate, and seven or eight days in a cold climate. Canal has found it living in charbonous meat after eight days; Nocard has likewise found it living and active after 17 days in the carcase of a guinea-pig. When the surrounding temperature attains

18°, 20°, 25° C., the cadavers preserve their virulence for a much shorter period. In addition to this, all things being equal, the virus preserves its life for a much longer period when the carcase containing it has been opened and cleaned. It preserves itself equally in cadaveric débris in the charbonous products, in the dejecta and excretions of the sick, in the urine and fæces; putrefaction causes them to lose their virulence. But, thanks to the presence of air, spores may form in these various products, and in that case, of course, the virulence persists for a longer time. The virulence of charbonous meat may or may not be destroyed by salting (Peuch); after a thorough salting it may be, but after an incomplete one it may preserve its vitality.

If the bacilli do not form spores in the cadavers, as we know they do not, when they do not gain access to the air, still those which are rejected from the mouth, nose, and anus in the blood which usually flows from these natural orifices, also those in the urine and excrements, gaining, as they do, exposure to the air and oxygen, go on to spore formation. The bacilli are often exposed to the air in this manner, or by skinning the carcases before burying them, or by allowing the blood and excretions to remain on the latter, etc., and spore formation takes place. The spores, once formed, preserve their vitality and their virulence for years; they will remain latent, but capable of development when introduced into suitable surroundings,—such as, for instance, the body of an animal susceptible to the malady; and hence it is that we are constantly observing the appearance of charbon in animals pastured on land where animals have died of the disease, or where they have been buried, or on land which has been manured with manure from stables, etc., where anthrax has been prevalent. Besides this, by cutting up a portion of a charbonous organ, such as the spleen, into fairly thick pieces, and drying it at a temperature of 25° to 35° C., the rods may bear spores which remain preserved for along time, and are, if anything, the more resistant the more they become dried; whilst if we cut up the organ into finer portions, or if we rapidly dry it, the rods perish. From this we may gather what is likely to occur in the excrement or other products strewn on the ground, roads, etc. Virulence persists when spores have had time to be formed; it persists in the soil of ditches or holes, manure, urine, water, and on pastures.

When we mix charbonous blood with the earth experimentally the microbe lives and multiplies if the temperature is propitious, and more especially so when the ground is watered with urine, etc. Spores are formed and may be found several months afterwards in the soil after numerous changes of atmosphere and temperature. These spores being sown on a new soil, and this again in another (successive cultivations) the last one may produce charbon.

Pasteur and Schrakramp have observed that the bacillus runs through the whole course of its development in the soil. It is very probable that spores sometimes cultivate in stagnant water rich in organic matter. We have observed the presence of charbonous germs in water where they have been carried by the rain.

It has been perfectly clearly demonstrated by experiment and by observation that charbonous germs are preserved in the soil. "By washing suspected earth and allowing the water to settle," says Pasteur, "then submitting the deposit so obtained to a temperature of 90° C. for some minutes, we destroy all the germs excepting the spores of anthrax or septicæmia, if there are any; by inoculating animals with this deposit we get in some septicæmia, and in others charbon. In this manner the presence of charbonous germs has been detected in earth from graves three to twelve years after burial of animals suffering from charbon; animals have been seen to contract charbon after having eaten herbs, etc., grown on graves two years after burial; forage deposited on soil containing spores of charbon has been observed to commuicate the malady. Spore formation occurs more especially in the superior layers of the soil, but it may occur also in the deeper layers when the soil is permeable and the temperature favourable; Soyka has noticed that it occurs more rapidly in a humid soil than even a culture medium. it does not occur in water deprived of organic substances (Rivolta), but the spores are preserved in the water. In the same manner, according to the researches of Kochstetter, whilst the bacillus is preserved three days in distilled water, in ordinary water the spores remain for more than 154 days; according to the researches of MM. Straus and Dubarry, the bacillus may exist for two months in water, and the spores more than 130 days. To sum up, these charbonous germs preserve their vitality for years in the soil which covers charbonous animals' graves, and in the soil surrounding them; this soil may communicate charbon twelve years after the burial of the carcass, and perhaps even a longer time. We may discover germs in such graves, and may by intravenous injection of rabbits, guinea-pigs, or sheep, which are not easily susceptible to septicæmia, with the residue of the deposit from water with which they have been washed, induce charbon and thus verify the suspected material.

Although the cadavers have been buried at a good depth, charbon germs, despite the filtering action of the soil, remount to the top of the graves, where we find them, as has already been seen; Pasteur, Feltz, and Bollinger contend that they are brought to the surface by means of earth-worms, but this view is denied by Koch, who claims to have proved to the contrary by experimentally burying earth-worms in earth containing the spores, and on careful examination of the casts of these worms, finding no trace of charbonous germs of any kind. Pasteur,

Feltz, and Bollinger, however, state that worms proceeding from charbonous graves deposit in their casts, and especially those found in spring and autumn, such a number of these germs that it is sufficient to inoculate the deposit from the water with which these have been washed to produce the disease. Pasteur has been able, by employing certain antiseptics which have little or no effect on the virus of charbon, but which destroys a great number of other germs contained in earth, to cultivate and obtain a pure culture from the casts of worms.

Pasteur, and later Feltz, by mixing the bacillus in earth and causing worms to live in it, have observed after some days that the casts extracted from these worms contained the germ of the disease, for they gave it on inoculation, and it was possible to cultivate it from them. It has been in addition to this remarked that on soils in which worms do not live, such as schistous, granitic and chalky, charbon is unknown, whilst where the soil is clayey, loamy or alluvial, it is common. On the other hand, it is very natural to suppose that worms are not the only means by which charbon germs are brought to the surface; all creatures which live in the subsoil may bring up the germs.

The germs so brought to the surface of the soil spread about attaching themselves to plants; they may be taken up with dust, by the feet of animals, penetrate into the respiratory passages, attach themselves to the skin, hair, or wool; they may be carried by rain and deposited at some distance; they may be carried into streams, ponds, pools, tanks, swamps, deposited on their banks, and thus have more opportunities of penetrating into the organism.

These facts explain the following, which have been frequently observed: the frequent and successive appearance of charbon in animals which are pastured on tainted pastures; the cessation of danger in certain tainted pastures after a certain time; the infection of certain pastures, which not having previously been dangerous, suddenly become so; the appearance of charbon on soils where charbonous animals have never been buried; the appearance of charbon in inundated pastures, etc., etc.

Besides this, other circumstances than the burial of charbonous carcasses may facilitate the dissemination of germs. The bacilli may, as has already been explained, form spores in the blood which has escaped from the sick animal, or from the cadavers, in the dejecta, manure, urine, and various other matter proceeding from charbonous cadavers, and it is easy to conceive that there are numerous circumstances which favour the dissemination of germs, more particularly when we consider that the germs may multiply and form spores when they arrive on vegetable detritus, marshy ground, etc. So it has been observed that animals contract charbon when pastured on marshy ground or land subject to

inundation; animals placed in folds or stables, the floors of which have been taken from anthrax graves; animals pastured on lands manured with litter from charbonous sources, and those marching on roads over which charbonous animals have been travelling. Nocard and Jouanne have seen the importation of manure, especially artificial manure in the form of dried blood, lead to an outbreak of charbon. In the same manner, charbon may be introduced into a locality where it did not previously exist, by the utilization of manure coming from a country where charbon exists, by the use of manure from horse slaughterers' yards when certain parts, such as the matter from the digestive canal, have not been sterilized, by the excrements of animals which have swallowed the germs without having become inoculated, etc. Slugs, for example, may swallow the germs of anthrax and pass them out intact, and other animals may doubtless do the same. Emigration of animals usually checks or stops an outbreak when the animals are conducted to non-dangerous pastures, but when emigration is adopted a danger of corrupting the new pasture is inevitable; the danger is considerably lessened by submitting the cadavers and the dejecta of animals, which fall sick after the emigration has been carried out, to disinfection or destroying them. When the germs exist at the surface of the soil, rain splashes them up on to the herbage; dust also carries them on to plants, and it is easy, therefore, to conceive that herbage and forage may be infected on tainted ground and thus rendered exceedingly dangerous; we have also seen lastly that water may contain and spread the contagion, giving rise to charbon in animals which drink it.

Contagion how produced.—Knowing what has preceded this, it is perfectly easy to understand how charbon appears and is propagated. The origin of the affection is always the introduction of the bacillus or its spore into the organism, and it is consequently of importance to determine the principal circumstances under which it propagates itself. The contagious nature of charbon, its propagation by contact, is clearly shown, as we have seen previously, by numerous observations and experiments with *sang de rate* of the sheep, charbonous fever of the horse, splenic apoplexy of the large ruminants, and malignant pustule in man, which are of the same nature and may be transmitted by inoculation.

Contagion may occur directly from the sick animal to a healthy one; this method of contagion is more frequently observed than is indirect or mediate contagion.

Direct or immediate contagion.—Direct transmission sometimes occurs from the mare to the fœtus, or from a sick animal or cadaver to the man who is attending to or working with it.

Intra-uterine transmission.—Brauell, then Davaine and others as well, have observed that charbon is not transmitted from the mother to

the fœtus, and that the blood of the latter is not virulent, nor does it contain rods, when borne by a charbonous mother. They consequently were led to the belief that perfect filtration occured in the placenta, the rods being arrested by the flexuous capillaries there. However, it has been for some years established that this rule is not an absolute one, but is subject to some exceptions. Although generally we do not observe the presence of rods nor the existence of virulence in the blood of the fœtus, although sowings made with the fluid often remain sterile, there are numerous cases, varying in frequency with the species on which the observations are made, in which, by a bacteriological examination, by inoculation, and more especially by culture, we may observe the presence of the microbe in the organs or the blood of the young creature. Toussaint, in verifying whether the blood of the fœtus of a female dead of anthrax conferred immunity, caused the death of a sheep by the inoculation. There had then in this case been intra-uterine transmission, but as the autopsy of the dead sheep had been made somewhat late, Toussaint considered that the germs had passed through the placenta after death.

Messrs. Strauss and Chamberland, in 1882, observed that in acute charbon in the female guinea-pig, the placenta often allows the microbe to pass through it, and that the blood of the fœtus becomes in this manner virulent. Females inoculated at various periods of gestation and dying of charbon, the blood of the fœtus was examined microscopically, inoculated into animals, and sown in broth. The microscopical examination did not as a rule reveal the presence of the bacillus on account of the few numbers in which it was present in the blood Cultures were fecundated sometimes with the blood of various fœtuses; sometimes with one, two, or more; at other times it occurred that of several flasks sown with blood from one fœtus, some cultivated, whilst others remained sterile. On inoculation it was found that a negative result followed the inoculation of the cultures which remained sterile; sometimes also a similar result was obtained with blood which had cultivated in broth. The result of these experiments shows that culture is the most certain method of revealing the presence of charbonous microbes in the blood or tissues of the fœtus, and also in media containing them in small numbers, and that we should not hesitate to make cultures with a large amount of seed in such cases.

During the same year, 1882, Perroncito showed that the passage of the microbe of anthrax from the mother to the fœtus might often occur in the guinea-pig.

Other experimentors have made similar observations. Koubasoff has drawn the following conclusions regarding the passage of the bacillus from the mother to the fœtus: "The bacilli pass from the mother to the fœtus,

and are, if anything, more numerous in its tissues the longer the mother survives the inoculation." Malvoz has also observed that the bacillus may in the guinea-pig pass from the mother to the fœtus, and he has decided that the passage occurs by means of small vascular ruptures of the placenta. Birch-Hirchfeld and Rosenblath have both observed the infection of the fœtus through the placenta, one in a pregnant rabbit and a goat, and the other in the guinea-pig; and they have explained the passage of the bacilli, as Malvoz has done, by hæmorragic lesions occurring in the placenta. Pangalli has found the bacillus in the blood of the fœtus of a woman dead of anthrax.

Generally, however, intra-uterine transmission of the bacillus does not occur. When it does occur, the bacillus appears to accumulate, more especially in the liver of the young animal.

To sum up, then, the bacillus of anthrax may pass from the mother to the fœtus, and in this way possibly may be explained certain cases of immunity of lambs born of mothers which have been affected with anthrax during gestation, or which have been vaccinated during that period. In the same way may be explained cases where such immunity has not been conferred, which is due to the passage not being constant. So also may be explained the cases of abortion which sometimes follow vaccination in the sheep and cow, and which are probably due to the contamination of the fœtus by the vaccinal fluid which destroys it whilst the more robust mother survives.

Transmission by contact.—When the milk becomes virulent the young may be infected by sucking; authentic cases of this method of contagion are, however, not forthcoming. Is this because the digestive organs of the young animals do not present any abrasions, etc.? Transmission by contact occurs in men working on cadavers or charbonous débris, who wound themselves during such operations, or who have already excoriations on the hands, etc. Such transmission has been frequently observed, and it is the most frequent method by which man becomes affected with the disease. In order that it may take place, that the bacilli may be absorbed, it is necessary that the skin be wounded, excoriated, incised, punctured, etc.; for, when intact, it does not absorb charbonous microbes.

Indirect or mediate contagion.—Indirect or mediate contagion by the medium of a solid or liquid vehicle, or by the air; infection, in a word, by germs which are preserved in the external media, is the most frequent mode of transmission of charbon. It may occur in three principal ways: by the ingestion of food or drink containing the microbe; by the inhalation of dust, etc., mixed with the spores of charbon; and by contact of a contaminated body with the surface of the absorbents of the skin; by the stings of flies, and the like.

Contagion by ingestion.— Contagion is frequently effected by the ingestion of aliment or water contaminated with the germs of charbon. The mucous membrane of the digestive passages, whether it present abrasions or wounds, or whether it is capable of absorbing the germs, notwithstanding the fact that it is intact, often serves as the point of entry of the bacillus into the system. It has been stated that charbon, which seems to appear spontaneously in regions where it is enzootic, is often due to the ingestion of germs with the food, which are ordinarily absorbed by the lesions or excoriations of the mouth and pharynx, or in other parts of the digestive system. Besides this, the rôle of the digestive canal in contagion is well established by numerous observations and experiments.

Barthelmy has seen the horse contract charbon after drinking water soiled with charbonous débris. Renault had induced charbon in the sheep, goat, and horse, but not in the pig, dog, nor birds, by causing them to ingest charbonous materials; the pig, dog, and birds are ordinarily refractory when of adult age. M. Baillet explained the development of charbon in the mountain pastures of Auvergne by admitting that the animals contaminated themselves by ingesting herbs or forage or water contaminated with germs which are rejected from the bodies of the sick, and from cadavers badly buried, in quantity, and preserve their vitality in the external media in which they find themselves placed. Davaine induced fatal charbon in a guinea-pig by causing it to ingest charbonous flesh.

M. Decroix has observed that the ingestion of virulent material remained inoffensive. M. G. Colin has seen dogs ingest charbonous material with impunity, also pigs and birds which have a natural immunity; he has also observed rabbits remain unharmed although fed with the blood of a charbonous animal. M. G. Colin caused the gastric juice of dogs to act on charbonous material, and found that it did not produce charbon after inoculation, and hence concluded that charbonous material which entered the stomach was destroyed. But it has been observed that charbonous spores are found in active state in the excrement of animals which have swallowed them without contracting charbon; and, besides this, the spores or germs are often absorbed before arriving at the stomach, and beyond this if the bacilli under the form of rods are destroyed by the gastric juice, spores resist and may traverse the whole digestive tube without having lost their virulence.

The appearance of charbon in animals which have been pastured on fields where sick animals have previously grazed, and on places manured with manure from charbonous animals, has already been noticed; it has been observed in animals which have consumed herbage or forage taken

from fields in which, or near which, charbonous carcasses have been buried. Sometimes it has been observed that charbon has appeared in animals ordinarily refractory to the disease following the ingestion of charbonous meat, as in pigs, birds, dogs, lions in menageries, and even in man, etc. Bonnet d'Iseghem has observed the transmission of charbon to a person who had eaten cooked charbonous meat.

Pasteur has observed that sheep fed with aliment previously soiled with charbonous germs became in a certain proportion sick. He has observed that guinea-pigs fed on aliment with which the spores of charbon have been previously mixed contract charbon with difficulty. He has found spores in the excrement of sheep and guinea-pigs. He has recognized that we may considerably increase the mortality when we mix sharp-pointed materials with charbonous fodder—materials which are calculated to more or less wound or excoriate the digestive passages, especially the first part of these. In this way he has fed sheep with aliments watered with liquid of culture containing spores, causing charbon in a number of them; he increased the mortality by mixing with it thorns, barley awns, etc., capable of wounding the first digestive passages; he has recognized the fact that in these cases, as in cases of charbon naturally contracted, the same lesions show themselves in the mouth or pharynx, etc., and has consequently been led to the conclusion that absorption often takes place in these parts of the digestive passages. Toussaint, according to the lesions of animals dead of anthrax, has also come to the conclusion that the virus is absorbed in the commencement of the digestive passages. M. G. Colin having shown that following a charbonous inoculation the virulence shows itself successively, as do also the other alterations, in the ganglia situated in the course of the lymphatics setting out from the point of inoculation, Pasteur and Toussaint have recognized, by the examination of the cadavers of animals dead of charbon, that the virus is often absorbed in the mouth and pharynx, basing their opinions on the lesions observed in the neighbourhood in the ganglia. M. G. Colin observed the successive invasion with the virulence and morbid changes of the neighbouring ganglia. Toussaint observed the successive invasion by the bacilli, which he saw proliferate in a prodigious manner in the ganglia.

M. Rodet, basing his opinion on the state of the ganglia, was of the opinion that guinea-pigs which had ingested charbonous matter and contracted charbon therefrom, had done so when the digestive mucous membrane was wounded, the virus penetrating by the buccal mucous membrane. In the guinea-pig, as in the rabbit and sheep, there is no local lesion at the seat of inoculation; but we often find infiltration, œdema and tumefaction of the ganglia in the neighbourhood of the region inoculated; the lymph of the infiltration is, as we have seen, generally poor in bacilli; but the

tumefied ganglia s ft, infiltrated, and marked with blood-spots, are very rich in them. M. Rodet has besides noticed that guinea-pigs which have swallowed charbonous material have not contracted the disease; and he has noticed animals of the same species become charbonous from eating the cadaver of a guinea-pig dead of charbon, the osseous débris of which had doubtless wounded the bucco-pharyngeal mucous membrane.

In short, when we inoculate charbonous virus into the lingual, buccal, pharyngeal mucous membrane; when we chafe, wound, or otherwise injure these mucous membranes, and deposit charbonous matter on them, we induce charbon with initial lesions in the neighbouring structures.

Dr. de Maurans has also noticed the influence of the action of rough, pointed forage, etc., in the development of charbon; he has observed that in Provence charbon shows itself more particularly during drought, when the animals consume dry herbs or leaves of almond trees, which wound the mucous membranes of the first digestive passages. It has also been noticed that animals which are suffering from the lesions of aphthous fever are more prone to contract charbon. It has also been seen that in Beauce, the mortality is greatest when animals are conducted to graze on stubble fields, as they are then more liable to wound the mucous membranes, etc.

Perroncito caused "guinea-pigs to be fed with food mixed with the adult bacilli and saw only one become charbonous; having fed ten others with food sprinkled with spores, six became affected. From these experiments the following conclusions may be drawn: that following the ingestion of aliments soiled with the charbonous virus, infection may occur in the intestine; it is more difficult and rarer with the bacilli than with the spores; it may, however, occur when bacilli are included in pieces of meat and escape the action of the gastric juice, which is in reality a bactericide, and find parts of the intestine deprived of epithelium by the action of parasites or by the action of hard irritating portions of food; charbon which results from ingestion is generally due to the introduction of spores, but even the spores require for their absorption portions of intestine deprived of its epithelium.

The mucous membrane of the intestine, even when normal, may, according to Kitt and Koch, absorb the spores of charbon. Although the gastric juice generally destroys the bacilli it respects spores. Koch having caused animals to swallow spores included in a potato, saw that they died of intestinal charbon, whilst sheep fed in the same way on bacilli were not affected; whilst the observations of Toussaint and Pasteur tend to establish the fact that absorption occurs by the bucco-pharyngeal mucous membrane, or that it is favoured by wounds of various kinds,

Koch thinks that it occurs in the intestine, and that the spores fix themselves in the follicles or Peyer's patches and then gain entrance to the lymphatic system. Be whatever it may the real point of entry, there remains no doubt that the ingestion of food and water soiled with charbonous germs may induce the disease. It is, however, advisable to add that the ingestion of charbonous germs is not always followed by the development of the disease—far from it; we may even presume that the introduction of germs into the digestive passages is a rare cause of the disease in practice, either on account of the germs being ingested in insufficient quantity, or being attenuated, or being taken into the system of an animal already immune as the result of spontaneous vaccination at pasture.

To sum up, then, the development of charbon appears to be frequently due to the ingestion of forage, herbs, fodder, or drink containing germs. The digestive passages play an important part in the absorption of the bacilli; numerous experiments show that the ingestion of charbonous materials is more or less frequently followed by the appearance of the disease. Without denying that charbonous germs, and notably the spores, may be absorbed by the bucco-pharyngeal or the gastro-intestinal mucous membrane in an intact state, we must nevertheless admit that, according to the facts and experiments related, the penetration of germs by way of the intact digestive mucous membrane is difficult and rare. This penetration is facilitated, and the mortality increased, when the mucous membrane is excoriated, wounded, or removed, as occurs when animals are fed upon rough, hard, and pointed forage. It is demonstrated that absorption may occur in the mouth and pharynx when the spores are in the food or drink; inoculation and infection then occurs, as it does following an inoculation in the skin. It is equally demonstrated that it may occur in the intestine, so that we may get localization of the principal lesions in that organ.

Contagion by inhalation.—Infection may occur through the medium of the air; not that the air expired by sick animals carries the microbes, but because spores preserved on the surface of pastures, roads, etc., may be suspended by the wind, and may be thus inhaled and determine charbon following their absorption by the respiratory passages. Roche, Lubin, and Garreau have cited cases of contagion by the air. The Medical and Veterinary Society of Eure and Loir have observed that charbon may be transmitted by the medium of the air. Carmague has seen sheep become charbonous when inhabiting stables freshly cleansed. Pasteur has noticed sheep contract charbon by smelling or breathing air near the soil of graves where charbonous animals had been buried. Charbon also is more especially noticed in times of drought when the animals raise clouds of dust whilst travelling. Lastly, it has

been observed that cases of broncho-pulmonary charbon occur in man as the result of the inhalation of charbonous dust.

The charbonous virus may in reality be absorbed by inhalation; sometimes the germs may be arrested in the nasal cavities, trachea, bronchi, and be afterwards expelled with the mucus. Nevertheless the microbes may pass through the epithelium of these parts. Muskatblath has produced charbon by injecting the bacillus into the trachea. Buchner has obtained it by pulverizing in the respiratory passages, waters charged with the spores. Tchistovitch has produced charbon by the intertracheal injection of the bacilli.

Transmission through the skin.—The virus of charbon deposited on or placed in contact with the skin, may be absorbed when it is wounded, pricked, excoriated, or torn, when there is an accidental wound, or when it is scratched or wounded by bodies bearing the virus. In this manner animals pastured on stubble may get wounds on the limbs, and so the charbon virus may gain entry. In the same manner a grain of one of the Graminaceæ (*Stipa capillata*) introduced into the wool works its way into the skin, and, penetrating this, gives rise to an abscess through which charbon is said to be introduced. Proust saw a butcher who contracted malignant pustule by pricking himself with one of these seeds when working on a Russian sheep in Paris. In New Caledonia another of the Graminaceæ, the *Andropogon Allionii*, causes analogous and sometimes mortal wounds in sheep. Spear grass (*Andropogon*), babul, and ber trees are also liable to wound the skin in India and afford an entry to the virus. It has also occurred that charbon has been introduced into a farm and transmitted by shearers who had previously shorn the fresh skins of two sheep dead of *sang de rate*. Man has been known to contract malignant pustule by carrying the wool which he has been sorting to his lips. Also, both man and animals may contract charbon owing to their being inoculated by flies.

Transmission by the bites of flies.—The rôle played by flies, although exaggerated by the vulgar, deserves some attention, especially in India. Davaine and Raimbert have caused charbon by inoculating the material from the feet and suckers of flies which have been left under a watch-glass with charbonous blood, and although this does not prove that flies may transmit the disease by biting, it proves that they can carry the contagion from place to place; flies having a soft proboscis, those which do not bite, impregnate themselves with virus by alighting on charbonous material; when so soiled they may soil wounds, and perhaps in this manner give rise to charbon. As to flies having rigid proboscides, those which bite, as Hypoboscides, Stomoxyidæ, Tanystomata, Bombyliidæ, etc., they may doubtless inoculate an

animal with charbon after having bitten and soiled the proboscis with blood of charbonous animals. In this connection it may be remarked that in the Hôtel Dieu Hospital in Paris, M. D. Mollière had under treatment and cured a man attacked with anthrax (malignant pustule) following the bite of a fly which he had smashed on the bite. Besides, the intervention of the fly may be an occasional cause in certain cases, where, owing to the itching which it gives rise to, the person scratches and may thus inoculate charbon, or indeed any other virus, he has, on his hands or under his nails; this frequently happens to those who work with charbonous dèbris.

Whether the fly directly inoculates, or whether it simply gives rise to irritation, causing the person to scratch and inoculate himself, it is none the less true that it is commonly believed to be a source of transmission. It often happens, however, that local accidents of a different nature inducing malignant pustule are wrongly interpreted. It is not, nevertheless, impossible that flies soiled with the virus of charbon may become agents in its transmission. It may be taken as a general rule that the bites of most flies in this particular are of little danger, their bites being in themselves rarely dangerous.

In short, it is recognized clearly at the present day, and is a generally admitted fact, that the development of charbon in countries where it is endemic and propagates itself, is more especially due to the preservation of the germs from buried cadavers, dèbris, products and dejecta, etc. The germs may be disseminated and charbon imported into districts in various ways, by the movement of flocks or herds, by the transportation of forage taken from contaminated sources, by dogs, which, after having ingested charbonous material, may pass out the germs (spores) with their excrements, and thus contaminate the herbage on which they are deposited, or the water in which they drink after having made a meal off a charbon carcass, and jackals and other scavengers may do the same; or by water flowing, as in inundations; or by wool impregnated with dejecta or blood containing the virus, or by water in which it has been washed, etc., etc. Burials, clandestine, defective, not sufficiently deep, those made in places to which animals are permitted ready access, in earth containing many worms, etc., explain, together with the utilization of charbonous dejecta, the frequence and persistence of the disease. Burials of carcasses carried out in sandy, calcareous, and chalky soils are less dangerous, the germs in such cases not remounting to the surface, but remaining where deposited; but even in these cases the tops of graves are dangerous, because there is every possibility of the soil covering them being contaminated with germs which have escaped from the carcass.

Formerly the development of charbon was considered to be spon-

taneous and attributed to diverse influences. At the present day the expressions "spontaneous development," "spontaneous birth," are employed to express the natural development of the disease following reception of the germs from the media surrounding the animals. The causes cited formerly to explain the spontaneous development of the disease may be considered as simple circumstances favourable to contagion; they act by favouring the preservation and multiplication of the germs and their introduction into the organism. These causes are numerous, and it is at the present time easy to interpret their mode of action. For a long time an elevated temperature and warm seasons have been marked as favourable to the development of the malady, and more particularly have warm moist seasons been blamed; this is in reality due to the fact that a relatively elevated temperature and a certain degree of moisture favour the multiplication and development of the germs, the formation of spores, etc. For years it has been noticed that charbon more especially occurs at the end of spring or commencement of autumn, during the summer, during warm years, during warm seasons, tempestuous and humid, during warm succeeding moist weather, etc. Ponds, marshes, morasses, swamps, low-lying alluvial ground, sedgy vegetation, and stagnant water generally are cited as causes. All these favour the preservation and multiplication of the germ of charbon and its introduction into the organism; there is a propitious *ensemble* of conditions; a favourable medium which furnishes suitable aliment; contact of the air, and an elevated temperature, favourable to the formation of spores. Forage and water may contain the bacilli, and the animals by ingesting them may contract charbon. Dust may convey the virus and it may be thus conducted into the organism. All localities where charbon is prevalent contain in their soil, water, or the herbage which grows upon them, the germs of the disease. Places having a humid soil, a clay subsoil, favour the preservation and multiplication of the germs; and their waters, as well as their herbage, may communicate charbon. It has been pretended that changed aliments or foods in excess may cause charbon, but this has no trace of truth in it, provided that the foods do not contain the virus of the disease. Decomposd water cannot produce charbon if it does not contain the virus; and neither excessive fatigue nor insufficient food ever produce the malady of themselves. It has been remarked, as we have already stated, that charbon more particularly appears in rainy years, especially when the rains are followed by excessive dryness. The rain water dissolves a large quantity of organic and mineral materials which, when heat follows, constitute a favourable medium for the development and multiplication of the germs. We have also blamed damp habitations, badly kept and ventilated; but whatever be their defective hygiene,

they cannot cause the development of charbon if they do not contain the germs. Sometimes, however, as we shall see presently, debilitating influences play a certain part by inceasing the predisposition.

We may take it as a fact that anthrax, like charbon syptomatique, is a malady arising out of certain soils which are capable of preserving the virus. Its development, like that of charbon symptomatique, depends greatly on infection from the soil and infection by the soil; it appears more particularly when the humidity of the soil becomes lower after it has been subject to humidity and a sufficient elevation of temperature for the development of spores to take place.

Absorption of the virus, its mode of action, pathogeny.—A. *Absorption.*—The invasion of the organism by charbon microbes following their accidental or experimental inoculation or their introduction into the respiratory or digestive passages, is produced by a mechanism nearly always the same. When they penetrate in the form of spores these germinate and are transformed into rods; in all cases the germs are principally absorbed by the lymphatic system. They rapidly multiply in the lymph spaces and the lymphatic vessels; they quickly gain the neighbouring lymphatic glands and are there temporarily arrested, multiply, completely invade, and then pass beyond them, gaining, by means of the lymphatic vessels, other ganglia; and in this manner finally gain admission into the general circulation. M. G. Colin observes that the virus inoculated into the extremity of a hind limb causes engorgement, tumefaction, and assumption of virulence, firstly of the popliteal ganglion, then of that of the groin, etc., and finally the bacilli become spread through the general circulation after having multiplied in the lymphatic vessels; then the general symptoms appear,—engorgement and virulence of the opposite member and of the whole body.

As to the blood-vassels, it is not demonstrated that they do not absorb charbonous microbes, more especially when they are cut or opened by inoculation; it is not difficult to admit that we may arrest the development of the malady by dividing the lymphatics which carry the bacteria, or by extirpating the ganglia which receive them; moreover, we shall see later on that in man, cauterization or extirpation of the primary malignant pustule may contribute to recovery. In all cases the penetration of the bacteria into the venous circulation accelerates the evolution of the malady, if the number of microbes introduced is important. In reality, when the virus of charbon is directly introduced in any quantity into the vein (Delafond, Toussaint, etc.) it multiplies rapidly, and the subjects die more quickly than after subcutaneous inoculation; but when the number of the microbes introduced

into the vein is inconsiderable, their multiplication is slower and more difficult.

Be that as it may, the vessels preside over the absorption of the virus deposited in a wound, and the penetration of the microbes from the region inoculated may take place very rapidly, for, if five minutes after inoculation the inoculated part be amputated, it does not always stop the course of the malady, which clearly shows that a part at least of the virus has penetrated beyond the amputated portion.

According to M. G. Colin, the virus of charbon inoculated by subcutaneous punctures into the ear of a rabbit caused charbon and death, although the ear was amputated 5, 4, and 3 minutes after the inoculation. According to Davaine, the virus of charbon deposited in wounds on rabbits, of the extent of a shilling, made by excision of the tegument, did not generally cause charbon when the wounds were cauterized with sulphuric acid, etc., one hour after the inoculation, and hence it may be concluded that the nature of the wound influences the rapidity of the absorption. There is great irregularity in the duration of the absorption of the virus of charbon, even following inoculations by puncture (subcutaneous). M. G. Colin has observed that inoculation of the horse produces a marked local lesion, and that excision or cauterization of the tumour may prevent general infection. M. Rodet has observed that even in the inoculated rabbit the virus propagates itself with variable rapidity; sometimes excisión after 5 minutes have elapsed since the inoculation, does not prevent infection, whilst excision after from 1 to 10 hours stops it in certain cases. Moreover, excision arrests or does not arrest the infection, according as it is deep and well removed around the seat of inoculation.

The germs are taken up by the lymphatic vessels and multiply in the lymph, their propagation varies according to the conditions which the inoculated part is in, and according to the activity to the virus being greater or less. The rapidity of their absorption depends on the facility with which they gain admittance to the lymphatic system; in cases of accidental contagion they may remain for a considerable time at the point of inoculation before entering the lymphatics.

Incubation.—The time which elapses between the moment of the introduction of the virus and that of general infection varies from one to several days, according to the class of animal, the species, age, the state of health, the dose, and activity of the microbes. The development of charbon may be hastened by numerous influences, such as youth, extirpation of the spleen (Berlach), bleeding (Rodet), cold (Pasteur), method of alimentation (Feser), fatigue, privation, etc.

Phagocytism.—Resistance of the system.—The organism, whatever

it be, and whatever be its degree of susceptibility, offers a certain amount of resistance to charbon and to other microbes; it acts against them through the medium of its cellular elements and its fluids. Fodor has clearly shown the bactericidal rôle of the blood of the rabbit in the case of the virus of charbon; he has observed that these microbes, injected into the veins or sown in fresh blood taken from the animal, are destroyed in large numbers. The susceptibility of the rabbit to charbon after intravenous injection is explained by the localization of the bacteria in the parenchymatous organs, where it can develop free from the harmful influence which the blood exerts upon it, and finally invade this medium. Nuttal has likewise observed the bactericidal action of the blood, either normal or defibrinated, of the rabbit, mouse, pigeon, sheep, dog; of the aqueous humor, the pericardial fluid of the dog and rabbits to the charbon virus. Buchner also has recognized it in the blood of the rabbit and dog; and he attributes it to the serum itself. Wurtz has besides observed that the bacillus anthracis, either in the state of rods, filaments or spores, is destroyed after an hour's sojourn in white of egg at 38° C. Behring, Nissen, Metchnikoff and Roux, etc., have noted the bactericidal properties of the blood of the rat. On account of this bactericidal property of the blood and other fluids acting against the bacteria, we may explain why a small dose of the virus may fail to produce the disease, whilst a large one may do so.

The bactericidal action of the serum of the blood is aided in its antimicrobian work by the intervention of the cells of the tissues, more particularly that of the leucocytes which play the part of phagocytes. The spleen, according to the observations of Bardoch, producing phagocytes, exercises a great influence; it plays an important part, protecting the blood against the invasion of microbes. In 25 dogs deprived of their spleens, 19 succumbed to charbon, whilst 5 only in 25 animals with the spleen intact contracted the disease. Again, whilst normal rabbits bore the anti-charbon vaccination by Chamberland and Roux's method, which will later be described, rabbits, whose spleens had been removed, died in the proportion of 26 to 35 from the first vaccine.

Leucocytes act not only against the adult bacilli, but also against the spores.

Smirnof, Bitter, Lubarsch, and Fischer have observed that the bacilli may preserve their virulence for several days in the animal tissues of immune animals, such as sheep and frogs. From the experiments of M. Trapeznikoff recently published, we may draw the following conclusions; in refractory animals the amœboid cells, the leucocytes accumulate at the seat of inoculation and devour the spores, or ingest

and transport them to the various organs which exercise a bactericidal or sporicidal influence such as, if not to actually destroy them, renders their germination difficult; when the spores have had time to germinate before being taken up by the cells, the bacilli to which they give existence are seized and destroyed by leucocytes; the spores taken up remain for a long time alive and virulent, and if the phagocytes which have taken them up weaken and die, they germinate, become transformed into filaments, which may in their turn be taken up and destroyed; in animals not refractory, the spores are seized by the leucocytes in the same manner, but they germinate and destroy them.

Manner in which the bacilli, etc., act upon the system.—The bacillus, introduced into the system, develops and multiplies, determining death in various ways. It lives and multiplies at the cost of organic liquids; it lives and multiplies in the blood, after having first invaded the lymphatic ganglia and system lying between the seat of its entry or inoculation, and the confluence of the lymphatic with the circulatory system; it often forms a felting, more or less compact, in the ganglia and in the blood-vessels; it occasions vascular ruptures and more or less numerous hæmorrhages, by reason of the emboli which it forms and the diastatic matter which it secretes, which acts on the tissues, softens the walls of the vessels, etc.

The bacilli being ærobic, requiring air, absorb oxygen and give out carbonic acid in its place; they abstract oxygen from the red corpuscles, which being also ærobic retards their development; they finally, by abstracting oxygen, render the blood progressively asphyxic and unfit for the proper nutrition of the tissues. The black colour which the blood assumes is explained in this manner, and also the deep tint of the tissues which has earned for the malady the name of charbon. This asphyxiating action, however, does not suffice to explain death, taking into consideration the fact that the bacilli are often found in very small numbers in the blood, and that it has undergone other changes.

The bacilli becoming numerous in the vessels occasion real embarrassment to the circulation, if anything more when the blood becomes viscous; they form emboli in the vessels, diminish the fluidity of the blood, render the globules more sticky on account of a diastase which they secrete, determine vascular obliterations and hæmorrhages (Toussaint). Similar lesions are produced in the various species of animals; they are particularly remarkable in soliped animals; they are accompanied by voluminous œdemata in the neighbourhood of the ganglia, and collection of fluid in the serous sacs, etc., In certain charbonous animals the bacilli may take up their abode in the pia mater, become very abundant in the vessels, and determine death by causing vascular

alteration in the nervous centres; hence it is indicated that they should be searched for in these organs when the animals have shown sign of cerebral localization of the malady; this mode of action, as is the case with the preceding one, does not always suffice to explain death.

It is more particularly by producing a veritable intoxication that the bacteria determine death. The microbes of charbon in fact secrete toxic matters. Charbonous blood previously filtered and then mixed with normal blood, renders the globules of the latter more agglutinative (Pasteur); this implies the presence of some diastase. Besides this, according to Toussaint, we produce a local inflammation in animals which are refractory to the disease with filtered blood, which proves the presence of a soluble pathogenic substance. Hofa has found a very active ptomaine in cultures of the virus made on meat. M. Chauveau, by inoculating 100 grammes of the blood of a sheep dead of charbon, has caused rapid death of the inoculated sheep which was previously vaccinated; death was due to veritable poisoning, and there was no multiplication of the germs in the blood. M. Gamaleia, by treating the spleen of a charbonous animal with strong alcohol, and afterwards removing the coagulum with water, has obtained a liquid which caused high fever in the rabbit.

To sum up, then, when an animal has become accidentally or experimentally inoculated, the bacilli multiply at the seat of inoculation, and secrete diastatic and toxic products. They set up irritation, inflammation, exudation, local infiltration and œdema which serves as a culture medium for them, and extend towards and about the ganglia where the lymphatics come off. Bacilli pass into the lymphatic vessels; the microbes soon arrive in the neighbouring ganglia, set up inflammation, multiply, then pass into the efferent vessels and arrive at a second ganglion, and finally are conducted into the blood-stream; arrived in the blood, they multiply and go no further, excepting when vascular ruptures occur; it is on this account that if we inject the virus into a vein, animals die without presenting other lesions than those of the vessels; and also that physiological secretion, such as bile, urine, milk, the products of glands, do not contain the germs unless as the result of vascular ruptures; in fact charbon appears as an intravascular blood disease. But vascular lesions in the form of ruptures are common in charbon, more especially in solipeds, and the ox and sheep, etc. The circulation is impeded in the capillaries by the accumulation and felting of the bacilli and by the viscosity of the blood; the bacilli form agglomerated masses which obstruct the circulation in the tortuous capillaries. In the rabbit they pass through vessels, which easily rupture; in the sheep, ruptures are produced in the kidney and intestine, whilst in solipeds ruptures are numerous and occur in various organs, the serous

membranes, in the connective tissues, etc. With these changes, the asphyxiating supply of bacilli increases more and more, as does also the sum of their diastatic and toxic secretions, and death occurs more particularly as a consequence of poisoning by these.

Degrees of Receptivity—Predisposing causes—Immunity.—Charbon may attack the various domesticated animals, and likewise man; but the receptivity varies with the species, individuals, the age, mode of entry of the virus, and the quantity and quality of the virus absorbed.

Herbivora—the sheep, goat, ox, horse, stag, and deer—are endowed with a great receptivity to the virus of charbon. Viewed from the standpoint of the occurrence of spontaneous charbon, sheep and goats come first, then the large ruminants and solipeds. The small domesticated animals, such as the rabbit and guinea-pig, are very susceptible, and although they are not as a rule exposed to conditions which render the spontaneous development of charbon likely, they contract it very readily when inoculated. The sheep and the horse take inoculated charbon readily: the ox shows a greater resistance.

Camels contract the disease, it is said. As regards the rabbit, guinea-pig, and mouse, nothing is easier than to infect them experimentally by subcutaneous inoculation; in ten to fifteen hours a local œdema, accompanied by an elevation of temperature, appears; the inoculated animals, apart from these symptoms, seem to be in perfect health until a short time before death, which occurs in 36 to 48 hours in the guinea-pig and 48 to 60 in the rabbit. Some moments before death the animals become agitated, respire quickly, fall into a state of coma, and succumb after some convulsions with notable lowering of the temperature. During the last moments of life bacilli appear in the blood, and at the autopsy, besides the ordinary signs of charbon already mentioned, we always observe at the point of inoculation the presence of a gelatinous exudation, transparent, sometimes faintly red-tinged, containing ordinary rods, in fewer numbers, but longer than those met with in the blood.

The pig is generally endowed with a very slight receptivity; he may occasionally contract charbon experimentally or accidentally. The carnivora—dogs and cats—are also less apt to contract charbon spontaneously; cases of accidental contagion have nevertheless been recorded, and the transmission of the disease by inoculation has been obtained. As to birds, their receptivity is still less; it is not noted in them as occurring by natural infection, and it is only by submitting them to experimental inoculation, under special conditions, that we can produce charbon in them. Lastly, man may be infected in different ways as has already been shown.

If such is the influence of species, it is necessary to add that it may be modified more or less by influences, which remain to be examined, by race, individuality, age, mode of inoculation, by certain debilitating influences, and by the quantity and quality of the virus.

Race may exert a considerable influence, rendering *quasi*-refractory animals belonging to species the most predisposed. So it is in the ovine species, the Algerian races offering a natural resistance to charbon. Algerian sheep were introduced on this account to the shores of the Mediterranean. This peculiarity of the Algerian sheep, well known to breeders in the south-east of France, has been verified experimentally by M. Chauveau. However, the resistance of Algerian sheep is not always absolute; it may be overcome by the introduction of a strong dose of the virus (Chauveau). From observations made it appears that certain of these sheep contract spontaneous charbon, and also that the *quasi*-immunity becomes much weaker, and finally lost in those animals which are imported and reared in France. Also, according to M. Chauveau, French sheep imported into Algeria do not appear to acquire a like immunity, and it is therefore beyond doubt that the immunity is due to race. The Indian sheep is very susceptible to the disease. Horses, which in Sardinia, Russia, Siberia, etc., are frequently attacked, are more rarely so in France and Central Europe. Toussaint says the ass, and Tayon more particularly the African ass, contracts charbon less easily than does the horse.

Certain subjects belonging to species the most susceptible, may sometimes present an individual immunity not becoming, infected in dangerous centres, escaping experimental infection by ingestion, and sometimes even resisting inoculation. This individual immunity may be due to the great resistance of the organism to the disease, or, better, to a certain degree of natural vaccination which the animals have undergone in the media where they live.

The age of animals contributes greatly to modify their receptivity; with a few exceptions it is more especially in youth that the contagium is virulent; young animals belonging to *quasi*-refractory species may be more easily infected than adults. In the same way young pigs ordinarily contract charbon after inoculation (Crookshank and Peuch); young birds and puppies may be infected in large proportion by inoculation (G. Colin, Œmler).

The mode of introduction of the virus may also exercise real influence upon the degree of receptivity of the animal. We may, for example, give fatal charbon to animals not naturally susceptible, by inoculating them subcutaneously with the virus; bovine animals appear to be less sensible to contagion by inoculation than to natural contagion, whilst horses, again, are more susceptible to inoculation than to naturally-

contracted charbon. Again, injection of the virus into the anterior chamber of the eye is more fatal in the pigeon than is subcutaneous inoculation. Numerous influences may modify the receptivity of the animals; these are for the most part influences which are of a debilitating nature. So, as Pasteur has shown, we may render the fowl susceptible by lowering its temperature; and Giber shows that, inversely, we may inoculate the frog successfully by raising the temperature. Sheep are, according to Rodet, rendered susceptible by bleeding before inoculation. Feser states that feeding on watery foods intensifies the action of the virus. Bardach says that ablation of the spleen, and the intravenous injection of inert powders, increase the receptivity in the dog. According to Charrin and Roget, fatigue renders white rats, which are often refractory, more apt to contract charbon. Canalis and Morpurgo state that inanition commenced some days before inoculation, or even afterwards, renders the pigeon susceptible to charbon, and it is the same with the fowl. Privation then may result, as I have observed, in many animals in increase or even creation of susceptibility. So also, according to Platania and Wagner, the introduction into the system of such substances as curare, alcohol, chloral, enables us to give charbon to the frog, fowl, and pigeon. So may previous disease or bad hygienic conditions increase the receptivity of animals.

The quantity and the quality or energy of the virus has a considerable effect on the susceptibility of individuals to charbon; the least susceptible animals may be rendered more or less gravely ill by the introduction of strong large doses of the virus, more particularly when it is very active. Algerian sheep, for example, may become charbonous after inoculation with very large doses of virus (Chauveau). To sum up, the differences which have been observed regarding the receptivity of animals relates to the species, race, individuals, age, state of health, hygienic conditions, the mode of entry of the virus, and its quality and quantity. This resistance is variable; it is more or less permanent and complete: it rarely, however, enables animals to withstand experimental inoculation. It is explained by the increase and diminution of the power of the phagocytes and the bactericidal state of the tissues; the influences which diminish it act by lessening these two powers.

Natural immunity leads us to the consideration of acquired or conferred immunity of vaccination, and the attenuation of the virus of charbon.

Attenuation of the virus of charbon.—We were led to the idea of vaccination and the enquiry into a mode of attenuation of the virus of charbon, by the observation of the non-receptivity of animals which had previously suffered from an attack of the disease.

M. Pasteur, after having observed that fowls fed upon food soiled with the material of fowl cholera, became vaccinated, endowed with immunity, and rendered refractory, when they did not die, also noticed that certain sheep which had ingested food soiled by the virus of charbon, escaped death after having been visibly ill, and that they afterwards resisted inoculation with charbonous material. He asked himself if it might not, under these circumstances, be possible to vaccinate sheep against the disease by previously submitting them gradually to the ingestion of food soiled by the spores of charbon (July 1880). After this, M. Pasteur observed, in September 1880, that the cow might recover spontaneously from charbon, and that once recovered she was vaccinated. Pasteur then came to the conclusion that ovine and bovine animals which had recovered from spontaneous charbon or from the inoculated disease were immune. M. Chauvean also noted the immunity of Algerian sheep and the immunity of lambs born of a mother inoculated with the disease during gestation.

Attenuation by heat.—M. Pasteur having succeeded in attenuating the virus of fowl cholera and transforming it into a vaccine, Toussaint attenuated the virus of charbon, and invented the procedure of attenuation by heat, and also that of attenuation by the use of an antiseptic. Inspired by the discoveries of Pasteur, who vaccinated the choleraic virus and found that the vaccinated fowl resisted cholera, Toussaint sought and found the first means of vaccinating charbonous blood by following a method different to that which had led Pasteur to vaccinate the virus of cholera, and which had been kept a secret. He defibrinated charbonous blood, passed it through linen, and then filtered it, after having diluted it, through twelve folds of paper; he inoculated the filtrate into a first series of animals, which it rendered seriously ill, but which recovered, and were found to be vaccinated. However, Toussaint, who essayed to vaccinate with a charbonous humor deprived of microbes, recognized that it was a dangerous method, as the filters of tenallowed bacilli to pas sthrough them, which destroyed the animals instead of protecting them. Filtration is in fact a defective mode of procedure. Generally, filtered blood gives, by inoculation, one or other of the following results: it induces charbon and destroys the animal; or it gives nothing, and does not confer immunity. We cannot obtain a charbonous blood so surely vaccinal by diluting it, as by filtration, which arrests the majority of the bacteria.

Toussaint, abandoning the procedure of attenuation by filtration, had recourse to the employment of heat. He observed a fact of great importance, which has served as a basis to the general method of attenuation of virus; he recognized that, by the employment of heat, we could diminish the power of charbonous virus to such an extent as to

be able to inoculate it without causing the death of the animal, and that we could at the same time confer an immunity against the attacks of a non-attenuated virus.

He obtained charbonous blood, suitable for inoculation, which did not produce charbonous fever in a fatal degree, but only a very slight attack, and which conferred immunity, by heating for ten minutes, at 55° C., charbonous blood which had been previously defibrinated. Scarcely had this mode of procedure been published than it was attacked by Pasteur, who was not, however, able to confute the fact that heat was capable of attenuating the virus of charbon. Toussaint was for the moment mistaken as to the explanation of the mode of action of heat and believed that the heating destroyed the bacteria; he attributed the immunity to the introduction into the organism of a material which was antagonistic to the development of the bacteria. In reality he was nearly, in so thinking, attributing the immunity which he had obtained to the action of heat in attenuating the virus.

Pasteur denied this explanation; he remarked that the bacillus could resist a temperature of 55° C. for ten minutes, and even for a longer time; he recognized that the bacilli were modified, and that it was not the soluble product formed by it during its life which was the cause of the vaccination in Toussaint's experiments; he observed that when heat was sufficiently applied to kill the microbes, as could be assured by culture, inoculation was not preventive. However, as we shall see later, the soluble products are capable of conferring immunity. Besides this, Trasbot had recognized that the blood of the fœtus of a sheep dead of charbon, which did not contain any bacilli, did not possess any vaccinal properties.

Pasteur recognized, in short, that in the procedure of attenuating by heat he might arrive at one of the three following results: frequently the virus, the bacilli, are killed by the heating, and inoculation does not give immunity; at other times the virus retains mortal virulence and inoculation kills; at other times, again, the bacilli are attenuated and inoculation may confer immunity, though the bacilli thus attenuated are not of the nature of stock, but may regain their virulence in the organism and in cultures.

Toussaint was then the first demonstrator of the possibility of preserving from charbon by preventive inoculations; he first succeeded in attenuating the virus and rendering it vaccinal, and although his method has not furnished altogether the practical solution of vaccination, it is none the less true that it has in the end done so after perfecting and development by M. Chauveau. On the other hand, the method of attenuation by heat offers immense advantages by permitting us to attenuate virus, the agent of which has not been determined. It has

been applied and has succeeded in giving vaccines with septic virus—the virus of charbon symptomatique, etc.

Toussaint, recognizing that his first explanation of vaccination by heated blood was erroneous, and having observed that filtered blood did not confer immunity, discovered at the same time the method of attenuation by the employment of an antiseptic. He conferred immunity by treating blood which had been defibrinated and passed through a linen or paper filter, with diluted phenic acid, one hundred grammes of blood to 1 or 1½ grammes of phenic acid dissolved in 5 cubic centimetres of alcohol and placed in 100 grammes of water.

The method of attenuation by the employment of an antiseptic was afterwards applied by MM. Chamberland and Roux to cultures of the bacilli: by the employment of a solution of phenic acid or bichromate of potash, they succeeded in attenuating the virus of cultures, and forming, as we shall see later, races of attenuated virus.

Attenuation by culture in the presence of air at 42°-43° C.—M. Pasteur, after observing the immunity of the sheep and ox to charbon, after they had been vaccinated with a culture of the virus of fowl cholera; after observing that sheep resisted the malady contracted by the ingestion of spores and acquired immunity; after noting that the cow frequently resists spontaneous or inoculated charbon and becomes refractory, applied the method which had served him for the attenuation of fowl cholera to the virus of charbon, and succeeded in attenuating it. Profiting by his experience with fowl cholera virus and also from the results obtained by Toussaint with heat, he invented his method of attenuation by the combined action of heat and air upon cultivated bacilli.

The bacillus, cultivated under ordinary conditions, multiplies for from 24 to 48 hours by scission, then it forms spores, which are henceforth little modified by contact with the air, and preserve all their virulence for a long time. But the bacillus, in its filamentous form, and so long as it only multiplies by scission, is attenuated by the action of the oxygen of the air; it suffices then to submit it to the action of the oxygen of the air, and to cause it to develop so that it cannot form spores; by this method of proceeding we are enabled to obtain the microbe of charbon in the most diverse degrees of virulence, from that of a virulence sufficient to destroy the animals which offer the greatest resistance to that which is almost, if not entirely, innocuous, passing through all the various degrees imaginable. The virus, in its various degrees of attenuation, may reproduce itself in new cultures which preserve the attenuated virulence, whilst that attenuated by heat at 55° C. does not retain its attenuation in cultures in which

it is sown. Amongst these attenuated bacilli of various degrees of virulence, it becomes easy to find one which, when inoculated into animals, only gives them a mild and benign attack of the disease, and confers upon them immunity against the non-attenuated virus.

At temperatures the most elevated compatible with the culture of the bacillus, varying with the medium used, they do not form spores. They do not grow at 45° C., but will at 42°-43° C., and at 42·5° C. they do not form spores in cultures made in flasks where the liquid is deep enough. Koch says that they do so when cultivated on plates.

The bacilli, cultivated in chicken broth, either neutral or feebly alkaline, in contact with the air, at a constant temperature of between 42°-43° C., do not even after a very long time form spores. Left at that temperature, in contact with the air, the microbe, after being multiplied by scission and forming a filamentous mycelium, becomes rapidly of less virulence. During the first days it passes through various degrees of attenuation. Each of these various conditions of virulence, more or less attenuated, may be reproduced by culture.

Each attenuated microbe constitutes a vaccine against the superior microbe; and it is easy to discover, in these successive viruses, vaccines capable of producing charbonous fever in animals without destroying them, and to confer such immunity on them as to preserve them from the mortal malady. Once attenuated, the microbe preserves its attenuation in the new cultures, and is sown in fresh media maintained at a less elevated temperature; it then produces spores of an attenuated virulence, from whence are produced again bacilli equally attenuated.

To recapitulate, the bacillus cultivated at 42°-43° C. in contact with the air, becomes progressively attenuated; each state of virulence is reproduced by culture, preserving its proper virulence; attenuated virus may produce spores similarly attenuated when placed in a medium the temperature of which is 33°-35° C. The spores which they produce are attenuated like the bacilli, and keep better. Each attenuated bacillus may be considered as a vaccine to the one superior to it in virulence; it gives a benign form of disease, when sufficiently attenuated, and confers protection against a graver attack: in this manner, by inoculating animals, first with a very attenuated virus, and then with a stronger one, we are enabled to confer upon them immunity against very virulent charbon without running any, or scarcely any, appreciable danger of causing serious illness, or destroying them. At the end of 12 or 13 days, the bacillus, cultivated and maintained in contact with the air at 42°-43° C., loses its virulence as regards the adult guinea-pig, and when inoculated into it does not kill it, whilst it lives and may be cultivated and reproduce itself in this inoffensive condition. At

the end of 31 days it only kills young mice ; and at the end of a month and a half it entirely loses its virulence. The microbe of charbon may be attenuated without any marked change in its morphological characters ; it may retain this attenuation in cultures, produce attenuated spores, give only a benign form of disease, and confer immunity. The attenuated virus presents a thinner, shorter, and more divided form of bacillus ; and the spores are very small. The virus attenuated by exposure to the air at a temperature of 42° to 43° C. is less abundant, and forms upon the sides of the flasks a uniform deposit, whilst the virulent virus forms a flocculent, snowy mass, made up of long filaments. When we sow attenuated spores, however, we obtain filaments forming flocculi. The attenuated bacilli and their spores are less stable, and perish after some months. The attenuation obtained by this method is due, according to M. Pasteur, to oxygen : he has recognized the fact that the action of oxygen varies with the temperature and with the culture medium ; he also recognizes the influence of the temperature, for he has seen the guinea-pig resist for five or six days inoculations made with a virus heated to 42°-43° C. in closed tubes. The virus attenuated by ten minutes' rapid heating at 55° C. does not preserve its attenuation in cultures in the same way as those attenuated at 42°-43° C. ; this is not due to a difference in the mode of action, but to the duration of the action of the temperature. The virus of charbon, of a very attenuated virulence, may regain it progressively, so long as it has not been killed outright, by successive cultures upon animals which are very susceptible and very young, then upon older animals, then upon animals less susceptible. If we commence upon guinea-pigs of 1, 2, 3, 4, 5, 10, 20, 30 days old, then pass on to those several months old, then to the sheep, for example, we resuscitate the virulence ; in this return to virulence we may, by cultivating virus at the various stages of the process, produce vaccines of the various degrees of activity. In the practice of protective inoculation, according to M. Pasteur's method, we employ successively two vaccines of different degrees of attenuation, commencing with a very attenuated virus, obtained by exposing culture at 42·5° C. for 15 or 20 days, and following this up with a second vaccine less attenuated, derived from cultures exposed to the air at 42·5° C. for 10 or 12 days.

In the vaccines prepared according to M. Pasteur's method just described, the properties acquired are not absolutely fixed. After a series of successive generations, or during the prolonged inactivity of an attenuated culture, the virulence may become modified, increase or decrease, and hence it becomes necessary to return from time to time to the commencement of the operations, and produce fresh vaccines in order to be certain of the results. We must never lose sight of the fact that charbon vaccines regain their virulence when they have

developed in a sheep or other animal, and have accidentally destroyed it after inoculation. After the publication of the results which we have set forth, the method of attenuation instituted by M. Pasteur was tested outside his laboratory; and these tests were fully confirmative (Feltz, Perroncito, etc., etc.); to-day the method is applied in various bacteriological institutes.

Attenuation in the soil.—M. Feltz, in testing the facts published by M. Pasteur and his pupils, after having observed that the virus cultivated in broth at 42°-43° C. becomes progressively attenuated, whilst it is not sensibly attenuated below that temperature, has, remarked that rabbits resist the attenuated virus better than guinea-pigs; that rabbits inoculated with a virus insufficiently attenuated for them, die,—some rapidly, others in from 8 to 10 days,—and that amongst these latter some die of charbon, presenting few microbes and showing hæmorrhagic spots on the gastro-intestinal mucous membrane, capillary emboli formed of bacilli, whilst others die after having passed through charbon, without presenting microbes even in the hæmorrhagic spots on the intestine. He concluded from these observations that in the case of spontaneous recovery, there is destruction and elimination of the virus by the digestive apparatus. He observed that earth rendered charbonous loses its virulence in time; that nature accomplishes attenuation of the virus in the soil analogous to that which is artificially produced in laboratories, and that it is in this way that we may explain the greater or less virulence of epizooties of charbon. The observations made by Feltz of the attenuation of the virus of charbon in the soil enable us to explain not only the variable gravity of epizooties of charbon, but also the more or less marked resistance of certain animals to the infection. It becomes certain, thanks to these observations, that animals may become vaccinated more or less completely in pastures by introducing into the system attenuated spores.

Attenuation by compressed oxygen.—M. Chauveau, having recognized the possibility of attenuating the effect of virulent inoculations by the employment of very small doses of the virus, after having demonstrated the fact that it is possible by this proceeding to increase the immunity of Barbary sheep, turned his attention to the attenuation of the virus of charbon. In 1882 he experimented with the procedure of Toussaint (heat); he gained his object and realized the explanation and development which it admitted of; he decided the conditions, the degree and duration of the heating necessary to attenuate charbonous blood; he showed by means of a more or less prolonged heating that we may obtain virus of various degrees of attenuation as by M. Pasteur's method. He advised, for practical purposes, that defibrinated blood be heated for fifteen minutes at 50° C. to produce a first vaccine (premier vaccin faible), and for nine or ten minutes to produce the second

vaccine (vaccin fort), and that we should inoculate animals first with the weak and later with the strong vaccines, allowing some days' interval between the inoculations as is done in M. Pasteur's method.

M. Chauveau besides demonstrated in 1883 that heat may be used to attenuate cultures of the virus rapidly; that cultures developed in 20 hours at 42°-43° C. may be attenuated in this way very quickly, in 3 hours at 47° C. they become so attenuated as to be innocuous to the adult guinea-pig; that we may produce attenuation without the aid of oxygen; that we may produce attenuated cultures capable of forming a stock and producing batonnets or rods and filaments equally attenuated, from the spores developed in it. By means of heat, M. Chauveau has been able to obtain an attenuated virus capable of transmitting its attenuation to the successive cultures which it serves to sow. It suffices in order to do this, firstly, to leave the cultures in broth sown with a drop of charbonous blood for 20 hours at a temperature of 42·5° C.; secondly, to place them after this time for three hours at a temperature of 47° C. to lessen its virulence; thirdly, to expose the culture so heated, or a broth sown with it, to a temperature of 35°-37° for from five to seven hours to allow of the formation of spores; fourthly, to heat the spore-bearing culture for a period of from one to one and a half hours at 80° C. The spores, already attenuated in a certain measure before this last heating, are still further attenuated to such a degree as to be innocuous to the sheep excepting in very exceptional cases; they are, at the same time, able to germinate in new media and to produce a mycelium attenuated like themselves. In order to obtain vaccines by this method of procedure, it is sufficient to make cultures in bulk, the first two as we have already described, and the third by sowing the attenuated virus in a large receptacle containing a considerable quantity of broth, assuring its rapid multiplication by allowing filtered air to gain access to the medium of culture; lastly, in the fourth, we give definite attenuation by a fresh heating. In order to do this and to ensure an equal attenuation of all the spores, we place the cultivation in tubes of 10 cubic centimetres capacity; seal them and keep them plunged for an hour in water heated to 84° C. for the first vaccine, and at 82° C. for the second.

M. Chauveau attributes the attenuation so obtained to the action of heat. He decides that in these cases oxygen has little, if anything, to do with the attenuation, and moreover states that it takes place more rapidly in vacuo than in the presence of air; that in M. Pasteur's procedure heat also plays the principal part. However, M. Chauveau realizes that the fixity of the attenuated virulence produced by heat is less than that produced by M. Pasteur's method, and that the attenuation produced by the combined action of air and a less elevated temperature (42·5°), but acting for a considerable time, is transmitted and

maintained better in future cultivations. That which the microbes have taken a longer time to acquire, they preserve better.

Having abandoned the procedure of attenuation by rapid heating as inferior in its results to the method of attenuation slowly by the combined and prolonged action of air and heat, M. Chauveau afterwards obtained results of greater scientific interest and greater practical value by cultivating the virus in contact with compressed oxygen. P. Bert had demonstrated that oxygen at high pressure, which respects the vitality of the spores, kills the adult microbes, and M Chauveau asked himself whether this gas, maintained at a pressure less than that at which it kills, might not comport itself as an agent of attenuation. He found that oxygen under pressure, and compressed air, could attenuate cultures of the virus and render them fit for vaccination. Air under moderate pressure augments the virulence of the microbes as regards the guinea-pig and sheep; under a little greater pressure it increases the virulence for the guinea-pig, but attenuates it for the sheep; lastly, under a pressure near that which arrests vegetation (13 atmospheres), cultures at 35° C. are sufficiently attenuated to not kill the sheep, but they still destroy the guinea-pig.

In this way, after having made four successive generations of a culture, leaving each generation for three weeks at a pressure of 8 atmospheres of air at a temperature of 38° C., M. Chauveau observed that the last generation, which still destroyed the guinea-pig, did not kill the sheep; a new culture being sown with this fourth generation and four new successive generations being obtained at a pressure of 9 atmospheres, we may obtain graduated vaccines of different intensity progressively decreasing. By employing pure oxygen, we may obtain the same result with a pressure five times less.

After these preparatory trials, M. Chauvea udecided upon the following *modus faciendi* to obtain charbon vaccine: sow several flasks with ordinary virus, and enclose them in a well-closed metallic receptacle; substitute pure oxygen for air, and accumulate it in the receiver up to a pressure of 2½ atmospheres, place the receptacle in a stove regulated to a temperature of 35°-36° C., and leave it to from 15 to 30 days, taking care to maintain the oxygen at a pressure of 2½; after the 15th day take seed from some of the flasks to fertilize large flasks in which at first a small quantity of broth is placed; leave these new cultures at the ordinary pressure of the air, and after several weeks add sterilized broth in order to dilute them. We obtain, in this manner, cultivations of various degrees of attenuation. We may confer immunity on the sheep by inoculating him with two drops of this diluted fluid, and on the ox by five drops.

In this manner, then, the attenuation obtained by cultivation under

compressed oxygen transmits itself to cultures of the second generation made at 36°-37° C. and under a normal pressure, and the cultivations attenuated in this manner may confer immunity upon the ox and sheep. More than this, the blood of a guinea-pig killed by this attenuated virus will be vaccinal for the sheep.

The virus of charbon attenuated by cultivation under compressed oxygen can confer immunity by a single inoculation. It is as innocuous as that of M. Pasteur; it preserves its acquired properties during several months,—for two months at least. However, its fixity is not absolute; after two months it has been found to regain a dangerous virulence. At the same time fixity of the attenuated virulence is not possessed by the vaccines prepared by M. Pasteur. All the microbes of the same cultivation do not necessarily or simultaneously undergo the same attenuation; certain of them may retain sufficient activity to kill or to return to their primitive type. Besides the attenuation which it produces, compressed oxygen has the effect of weakening the toxicity of cultivations, whilst leaving them their vaccinal properties.

In the more recent researches of M. Chauveau in 1889, by submitting new cultures sown with old ones attenuated in the same way, to the action of compressed oxygen, it has been found that the virus may be despoiled of its virulence, whilst preserving its power of conferring immunity; that attenuated to such a degree as not to kill the guinea-pig, (an animal most sensitive to charbon), it may be still cultivated and produce vaccine which is effectual without endangering the lives of the animals inoculated; that it may, lastly, be reinvested with its virulence by cultivating it in broth to which the blood of the guinea-pig has been added, and placing it in rarefied air.

If the blood added to the broth has been taken from a guinea-pig, the bacillus recovers the property of mortally infecting first a mouse and the newly-born guinea-pig, then adult guinea-pigs and rabbits. Arrived at this stage, the virus, in course of reconstitution, vaccinates small ruminants, being incapable of killing them. In order that it may attain this activity, we should cultivate it in broth to which the blood of the sheep has been added, the bacillus which is obtained is fatal to rodents. The types obtained in the ascending series may be reproduced with great constancy.

Attenuation by age.—In a cultivation of bacillus anthracis left to itself, the virulence disappears at the end of a longer or shorter period varying with certain intrinsic and extrinsic conditions which are not well understood. Whilst the virulence diminishes, the bacilli lose their power of vegetating and die. At a given time there remain in the liquid a few bacilli having still, but not intact, their virulence and power of vegetating. At this stage an inoculation performed in the ordinary way with the usual

quantity may be insufficient to betray the pathogenic activity still inherent to the cultivation. In order to reveal it, it becomes necessary to employ the method of cultivating it afresh or inoculating with a strong dose. In a cultivation, the various bacilli have not all the same virulence nor the same power of vegetating; time changes the weaker ones first, and by degrees the cultivation becomes poorer in microbes which are virulent and capable of growing; but if at a given time inoculation with a small dose is insufficient to betray the vestiges of virulence which remain, cultivation allows of our growing a generation of virulent bacilli. Analogous phenomena are produced in cultivations experimentally attenuated; and certainly we ought, in the study of the influences capable of restoring the virulence, to take account of a spontaneous selection of the virulent agents in the cultures of restoration. An ordinary simple cultivation may suffice to restore the virulence to an old culture (Arloing). Perroncito has succeeded in obtaining a charbon vaccine, capable of conferring immunity on the ox by a single inoculation, by cultivating the virus for five days, and then exposing the cultures to air heated to 20°-25°.

Attenuation by Antiseptics. — MM. Chamberland and Roux have, after Toussaint, attenuated the virus of charbon by the employment of antiseptic agents; they have been able to constitute races of attenuated virus by the employment of a solution of phenic acid, or of bichromate of potassium. These are the principal facts to be derived from their researches :—"The addition of $\frac{1}{400}$ phenic acid to veal broth stops all multiplication of the virus. Moreover, after a sojourn of 48 hours in such a medium, the bacillus has ceased to live; it gives no development when sown in neutralized veal broth. If the proportion of phenic acid is not greater than $\frac{1}{600}$, $\frac{1}{800}$, $\frac{1}{1200}$, the bacillus lives and multiplies; and, even after it has been maintained for a long time in contact with the antiseptic, it easily reproduces itself, when it is placed in a suitable nutritive medium. In this way, after six months the bacilli have remained alive in liquids at $\frac{1}{800}$ and $\frac{1}{1200}$ of phenic acid. If the dose of antiseptic is stronger, the bacillus dies more rapidly; in a flask at $\frac{1}{500}$ all life had ceased at the end of five months.

"It is sufficient to place $\frac{1}{800}$ phenic acid in the culture liquid to prevent the formation of germs. The bacillus dies in this medium without producing spores. When the dose of phenic acid is weaker, $\frac{1}{1200}$ for example, the filaments form spores.

The culture issuing from bacilli which have lived for twelve days in broth to which $\frac{1}{600}$ phenic acid has been added, is virulent to guinea-pigs and rabbits. The cultivation from the same bacilli, after two days, kills neither guinea-pigs nor rabbits. The action of the antiseptic has resulted in the diminution of the virulence of the bacilli. If we make

requent sowings from the original flask, we get a series of cultivations of decreasing virulence, which may furnish, as in the case of cultures at 42°-43°, attenuated virus capable of preserving animals into which it has been inoculated as a preventive, from mortal charbon. Repeated cultures of the vaccines reproduce virus with attenuated properties and perpetuate them. The filaments obtained in these conditions, in place of being abundant and long as in normal cultures and matting themselves into flocculi resembling cotton-wool, are more scanty in numbers and shorter, and deposit themselves in grumous masses on the walls of the receptacle. These bacilli, so altered in their form, easily give numerous and resisting spores.

"Phenic acid is not the only agent which gives these results; with bichromate of potash we may obtain analogous ones. A broth, to which has been added 1-1000-1-700 of bichromate of potash, will not cultivate the virus, which dies quickly in such a medium. A weaker dose of bichromate of potassium, 1-2000-1-5000°, allows of the development of the bacillus; but, under these conditions it does not form spores, and soon loses its virulence to such a degree that sown three days after the commencement of the experiment, it gives a cultivation which kills rabbits and guinea-pigs, but only a small percentage of sheep into which it is inoculated; a cultivation prepared from a bichromate broth, after ten days, still destroys rabbits and guinea-pigs, but does not kill sheep; lastly, after a longer interval, the cultures become innocuous even to guinea-pigs. Smaller proportions of bichromate of potash retard the formation of spores without absolutely preventing them. The bacilli formed from the filaments which have undergone the action of bichromate, form spores, which perpetuate their properties and assure their preservation. Moreover, if the action of the bichromate has been prolonged, the bacillus loses the property of forming spores. In this way, cultures issuing from a flask at 1-2000, after the 8th day from the commencement of the experiment, have never formed spores, and it has been the same with all successive cultures issuing from it. These bacilli, incapable of forming spores, inoculated into guinea-pigs, destroy them in from three to four days. A small drop of their blood sown in broth gives an abundant cultivation of bacilli, which does not form spores; they remain in the state of filaments, and after thirty or forty days they perish. "The diminution of the virulence of the bacilli so modified by means of antiseptics, is not a passing one; the cultivation does not regain its virulence. The bacilli, so attenuated by the action of antiseptics that they either do or do not form spores, preserve in repeated cultures a lessened virulence. It appears, therefore, that the varieties of bacilli so created are, if anything, more fixed in their new degree of virulence, as the action which has modified them is exercised more quickly upon them.

"Other antiseptics exercise an analogous action to those of phenic acid and bichromate of potash. On the other hand, the dose of the antiseptic necessary to produce a determinate effect varies with the composition of the culture medium. Each of the varieties of the bacillus has a special action upon the various species of animals. So that the bacilli attenuated by means of bichromate of potassium, may destroy sheep, or at least render them very ill, after which they are protected, whilst they do not produce any appreciable effect upon guinea pigs and rabbits, nor are they protected by the inoculation.

"In another series of experiments we have submitted the filaments to the action of a chemical agent in the midst of a liquid where its development is not possible; we have caused an antiseptic dissolved in pure water, which contains no nutritive element, to act upon the formed bacillus.

"The filaments of a drop of charbonous blood placed in water in which phenic acid 1-600 has been dissolved, perish rapidly; we have seen before that it lives and grows for a month in a nutritive medium which contains the same proportion of phenic acid. In a phenicated solution 1-900, the filaments remain alive for a very long time, as is proved by the fact that cultivations may be made from them after several month. During the whole time occupied in the experiment, they do not form spores, and their virulence becomes enfeebled. So that the cultivation of filaments which has remained for a month in contact with a solution of phenic acid 1-900 kills rabbits and guinea pigs. A cultivation made after three months contact does not kill rabbits. Under these circumstances, the loss of virulence is less rapid than is the case when the bacillus vegetates in the presence of an antiseptic. But a short time elapses between this diminution of virulence as regards rabbits and the death of the cultivation.

"The essential condition in the attenuation of the virulence of the virus of charbon, either by the method of cultivating at 42°-43° C., or by the employment of antiseptics, is the absence of spores in the filaments submitted to the prolonged action of the air, heat, or various chemical agents. The spore is the form in which resistance or permanence of virulence is most marked. It preserves, so to speak, from the action of the surrounding media, the properties of the filament which has given it birth. Despite this resistance to external agents, the spore may be modified and attenuated in its virulence like the filament itself.

"Well formed spores of the filaments, fifteen or twenty days old, are placed in contact with 2 % solution of sulphuric acid, and exposed at a temperature of 35° C. in closed tubes, which are frequently shaken to ensure thorough contact of the spores with the acid. Every two days a small quantity of these spores is sown in broth (veal) rendered slightly

alkaline. The cultivation so obtained during the first days kills rabbits and guinea pigs; the cultivation made on the fourteenth day only kills a small percentage of the guinea pigs into which it is inoculated. The bacilli, etc., so obtained form numerous spores, and preserve their attenuated virulence in successive cultures.

"But it is worthy of remark that cultures issuing from spores treated with sulphuric acid, which have lost their virulence as regards rabbits have not done so as regards sheep, and destroy them in the proportion of 7 to 10. This fact, and the analogous ones already quoted, shows that each species of animal has a particular receptivity for each of the races of virus which we have been able to prepare by artifices of cultivation.

"The diminution of the virulence of the spores and lastly their death under the action of sulphuric acid occurs, if anything, more rapidly as the temperature is more elevated and the acid more concentrated, and more slowly as the temperature is lower, and the acid solution more diluted." Zagari has attenuated the virus, by making it live in a broth which has cultivated the microbe of fowl cholera, and Pavone by cultivating it in a broth which had already cultivated the bacillus of typhus. In the same manner of working, we may also cite the progressive attenuation which Manfredi has obtained by cultivation in a medium containing a certain proportion of fatty material.

The bacillus anthracis sown in a medium composed of gelatine or nutritive jelly containing an emulsion 1/3 of its volume of fatty matter develops well and liquefies the gelatine. When more than one-third of fatty matter is present, the cultivation is slower and the bacillus forms long filaments with few spores; it ceases to liquefy the gelatine. When two-thirds of fatty matter is present, development does not occur. Simultaneously the virulence of the culture diminishes to its disappearing point; this attenuation depends upon the fatty matter introduced into the medium, for it augments and diminishes with the proportion of this material; but temperature and time have, at the same time, a great influence. So in the cultures of Manfredi, the virulence disappeared in two or three days at 37° C., in twenty or thirty days at 28°-30° C., in twenty-five to forty-five days at 19°-20° C. On the tenth day at 28°-30°, the cultivation killed all the guinea pigs, and the majority of rabbits inoculated; at the twentieth day it did not kill rabbits or large guinea pigs; beyond the twenty-fifth day it only killed mice; at the thirtieth day it lost all virulence. The attenuated bacilli preserve their degree of virulence when placed in ordinary media; more or less it has been noticed that in return cultures, a slight increase of virulence which has been only slightly attenuated occurs, and that there is a slight

diminution in those very much attenuated. These return cultures regain the property of liquefying gelatine, but they liquefy it more slowly than normal cultures. According to M. Duclaux, the attenuation so obtained is explained by the fact that the fatty matter employed in oxidysing impoverishes the medium in oxygen, saponifies and becomes acid.

Various means of attenuation.—M. Roux studying the action of the air and heat on the spores of charbon has recognized that they do not perish for a very long time at 70°, if they are protected from the air; and that they may after a time undergo a certain amount of attenuation before they perish. He has also found that under the same conditions as regards temperature, they die more quickly when they are heated in contact with the air, and that before perishing they undergo diminution in virulence.

M. Arloing has observed that the action of sun-light attenuates cultivations of the virus; and that it may be utilized in the preparation of the vaccines, if we are careful to allow it to act on transparent cultivations distributed in thin layers at the bottom of flat flasks. MM. Apostoli and Laquerriere have demonstrated that the action of a galvanic current may, according to its intensity, either kill the virus or simply attenuate it.

The virus of charbon may be also attenuated by its passage through various organisms. In this way, the virus appears to become attenuated according to certain experimentalists in a certain measure, by passing through the organism of the rat, white mice, rodents, frog, etc. It has also been thought that in a general way, the virus is attenuated in passing through the organism of an animal which is refractory, that of the fowl, pigeon, white rat, frog, etc. But it has been demonstrated that it does not attenuate in passing through the pigeon or rat (Metchnikoff) nor the fowl (Perroncito) nor the frog (Nuttal); it has also been observed that the virus of charbon cultivated from pigeon to pigeon becomes more virulent to the pigeon, fowl, guinea pig and rabbit.

Metchnikoff has observed that the bacillus cultivated in the blood of sheep previously rendered refractory, becomes in a certain measure attenuated. Sadowsky after inoculating charbon into the dog believed that he recognized some attenuation of the virus, but that when cultivated afterwards in broth it resumed its virulence. Malm has drawn the following conclusions from his experiments; the bacillus lived in the dog, an animal more or less constantly refractory to it, far from losing its virulence it increases it; the same phenomenon of increase of virulence is also observed in bacilli which have been passed through rabbits previously rendered refractory by

protective inoculation; the bacillus in passing through a refractory animal, increases in virulence and this increase occurs in the œdema following the subcutaneous inoculation, in the blood, and in the spleen after intravenous inoculation; in dogs which are not refractory the exaltation of virulence is irregular: a previous injection, either subcutaneous or intravenous, increases the natural immunity of the dog so as to render it absolute; dogs resist intravenous injection less than subcutaneous. Moreover, the bacillus does not appear to become attenuated by long residence in the organism of the frog (Lubarsch); it increases in passing through the ox and dog; also by being cultivated in a series of fowls of ascending age (Roux and Chamberland) by passing through the pigeon (Metchnikoff) as we have already seen; it increases rather than attenuates by passing through the organism of a vaccinated animal —the rabbit for instance. M. Nocard having injected the bacillus into the teat of a goat previously protected by inoculation has noticed that the animal did not become ill, although the bacillus multiplied in the udder, and he was able to obtain virulent milk for more than two months, but during the whole of this time the animal was in no way affected. To recapitulate then, numerous modes of procedure permit of attenuations of the virus; but at the present time two only are followed in the preparation of vaccines destined for protective inoculation, and these are those of M. Pasteur and M. Chauveau. We are able, by the methods of Pasteur and Chauveau, to obtain virus of varying degrees of attenuation capable of reproducing themselves with their newly acquired properties; some do not destroy the sheep, but still destroy the rabbit; others do not kill the rabbit, but are still fatal to the guinea pig, whilst others again do not kill the guinea pig, but destroy mice. We may gradually return these races of virus to their original virulence by cultivating them after a certain method of procedure, by inoculating animals which are still susceptible to their action, the mouse, and then animals more and more resistant, the young guinea pig, then the adult guinea pig, then the rabbit, and then the sheep. We may obtain vaccines which, inoculated without any great danger into animals, confer immunity upon them.

Pasteur's method of vaccination against Anthrax.—Charbon virus, attenuated by M. Pasteur's method, allows of our conferring immunity at once by a single inoculation, or progressively by several inoculations with vaccines less and less attenuated.

Method of operating.—(Condensed from Chamberland's "Le Charbon et la Vaccination charbonneuse.") In practice, vaccination is performed at twice, at an interval of 12 to 15 days between the inoculations; we inoculate to commence with a weak vaccine (premier vaccin) which only confers an incomplete immunity; and afterwards at an

interval of 12 or 15 days we inoculate a stronger vaccine (deuxieme vaccin), which completes the immunity. It is customary to make these two inoculations at different points on the body, the parts selected in the sheep being for the first inoculation, the inside of the right thigh, and for the second the inside of the left thigh, whilst in oxen the one is made in front of and the other behind the shoulder. In the case of horses, the inoculation is made in front of the shoulder in saddle horses, so that the point may not be in the way of the saddle.

Full grown sheep of all ages, lambs even when very young, ewes more or less advanced in pregnancy, some in the point of lambing, have been vaccinated without any untoward consequences. Still accidents have sometimes been reported as occurring in young lambs, when the same vaccine used at the same time in full grown sheep produced only a more or less slight disease, but not death. With the vaccines now in use even very young lambs can be vaccinated without inconvenience.

The vaccination of ewes on the point of lambing has also given rise to some accidents. Several times abortions have been reported to us. We thought at first that these accidents ought to be attributed more to the shaking which the animals had undergone in being turned over on to their backs in order to present the internal aspect of the thigh to the operator, but it may also be possible that the fever from which the ewe suffered may have directly induced abortion. According to researches, which have recently been made in collaboration with M. I. Straus, it appears that, contrary to the view hitherto held, bacilli introduced into the mother, sometimes pass into the fœtus. It must be asked, then, whether the cases of abortion which have been reported are not due to the vaccine bacilli of the mother, bacilli which she can withstand without serious results, passing into the fœtus, which being less resistant, has succumbed and has consequently been expelled. However this may be, it is better to vaccinate ewes when they are not pregnant, or at least during the earlier periods of gestation, except in urgent cases, as, for example, when the disease is raging in a flock.

It may be asked whether lambs born of vaccinated ewes, are themselves vaccinated. This question is not completely cleared up, and experiments are being made with regard to it. Everything leads us to believe that a large number of the lambs, if not all, do not enjoy immunity, so that for the present it is necessary to vaccinate lambs when there is reason to fear that they may be exposed to the spontaneous disease.

Spring ought, whenever possible, to be chosen for the operation, for the disease being rare at that time, we avoid, at least in the great majority of cases, the combined action of the vaccine and of the spon-

taneous disease. Morever, recently inoculated animals are better able to resist the disease which rages generally in summer and autumn. In oxen slight œdema sometimes follows the puncture: such swelling disappears spontaneously and should not be interfered with. Cows are also liable to suffer from œdema or even from perceptible fever after the inoculation; the quantity of milk diminishes; it is better therefore to vaccinate cows when they are giving little or no milk, and an additional reason why this is advisable is, that it has not been proved to be absolutely impossible for the vaccine bacillus to enter into the milk. All that has been said respecting pregnant ewes applies equally to cows in that condition.

The following are the instructions given for the guidance of veterinary surgeons in using the vaccine by M. Boutroux :—

Method of Operation.—*sheep and goats.*—The vaccine is sent out in tubes closed with caoutchouc corks, containing a sufficient quantity for the vaccination of 1, 2 or 300 sheep or 50, 100, or 150 cows, oxen or horses, the quantity used in the inoculation of the larger animals being practically double that for small ruminants. It is labelled, "premier vaccin" and "deuxieme vaccin." This liquid is to be introduced in a determined dose under the skin of the animals, and for this purpose we employ a Pravaz' syringe. The syringe must first be filled with the liquid. In order to accomplish this we first remove the fine wire from the hollow of the needle, and adjust the needle on the canula; the tube of vaccine is now well shaken so as to thoroughly mix the contents; unless this is done, the vaccine bacilli settle to the bottom of the tube, and there is considerable risk of drawing up several syringefuls of liquid containing few or no bacilli, whilst later we get a large number, with the result that some of the vaccinations will have a negative result. After shaking well then, the cork is removed from the tube, and the liquid is aspirated into the syringe by gently raising the piston. If the syringe is in proper order, it completely fills with liquid, leaving only a very small bubble of air under the piston, but it frequently happens that the sucker has become dry, or that the needle is not properly adjusted on the canula and then the liquid does not completely fill the syringe and a large bubble of air remains under the piston. In this case we must readjust the needle on the canula and reject the liquid into the tube, as it is absolutely necessary to have the syringe quite full, to the exclusion of all air. We perform the same manœuvre two or three times until the poiston becomes moistened, and if the needle is well fitted on to the canula, the syringe fills completely. This condition is, as has been already noted, indispensable. In cases where the piston has become very dry and allows air to pass, we may boil it in water, leaving it to cool in the vessel in which it has been boiled until moderately

warm, and then aspirating two or three syringes full of the water in order to cause the piston to swell. Water which has not been boiled must not be used for this purpose. It is of course of the utmost importance to employ antiseptic precautions in so far as regards the employment of a perfectly clean and sterilized syringe. We must also be extremely careful that we use every expedient to prevent the entrance of germs into the vaccine whilst aspirating it into the syringe. The syringe then being perfectly clean and filled with the vaccine completely, we turn the little nut which is at the top of the stem until it reaches the division marked I on the stem. Then an assistant seizes the sheep or goat to be vaccinated and presents the inner aspect of the thigh to the operator by turning the animal on its back in a sitting attitude. The operator now introduces the needle under the skin, towards the middle of the right thigh and presses the piston until the nut touches the syringe. The inoculation of the first animal is thus made. The syringe is now removed and the nut on the piston turned until it stands at 2. The second sheep is now inoculated and so on. One Pravaz' syringe holds enough for 8 sheep. The syringe is filled again and the process repeated. With a little practice 150 to 200 sheep can be vaccinated in an hour. After an interval of 15 days we inoculate them in the same way with the "dexuieme vaccin" in the left thigh.

Oxen and Horses.—We employ the same vaccine for these animals as for sheep and goats, but we introduce it in double the doses, so that one syringeful is only sufficient for four animals. In place of making the inoculation at the thigh we make it behind the shoulder, in oxen and horses. The skin of oxen being somewhat difficult to pierce, it is necessary to be careful to hold the needle exactly in the axis of the syringe, so as not to break it off. It is also a good plan to take a fold of skin in the fingers of the left hand to facilitate the introduction of the needle. The same needle which serves for the inoculation of sheep may also be used for oxen, but it is advisible to use a stronger one for large animals. M. Chamberland says:—The vaccine liquid introduced under the skin ought to be pure in order to produce its proper effect, that is to say, it ought not to be contaminated with any foreign organism derived from the ærial dust, morsels of wool or straw, or scraps of dung, etc., sometimes found on animals, for if such impurities get mixed with the vaccine they may give rise to other diseases, such as septicæmia, suppuration, etc. At other times these impurities may, as M. Pasteur has shown, produce no apparent effect, but may hinder the action of the vaccine. In order to obtain the highest possible degree of this condition of purity, the syringes are boiled after each operation, and put together again, in order to prevent the small quantity of liquid which has soaked into the piston or remained below from giving rise to new organisms; with regard to the tube

containing the vaccine, the caoutchouc cork by which it is closed ought to be carefully put back into the tube each time after the syringe has been filled, and only the extremity not directly in contact with the tube ought to be touched with the fingers. There is another cause of impurity which it is almost impossible to avoid. The outer surface of the needle, in fact, becomes quickly covered with scraps of wool, hair, or even of dung; in this way each time that the needle is put into the tube in order to draw up a fresh quantity of liquid some of these foreign bodies gets mixed with the liquid and destroys its purity; in consequence when but little fluid remains, that is to say, when the needle has been plunged into it ten times, the liquid is almost always impure.

We have endeavoured to obviate these difficulties and we believe we have succeeded in the syringe constructed under our direction by M. Colin. With this syringe once filled 50 sheep can be vaccinated in succession. The vaccine is not in contact with ærial impurities, and cannot be soiled by foreign bodies sticking to the outside of the needle. It can be easily cleaned.

Strictly speaking a single syringe of this pattern would do for all vaccinations; but it appears to be preferable to get two syringes, one for the first, and other for the second inoculation. This syringe has certain inconveniences; the liquid injected is not seen, and the quantity injected cannot be regulated with the same precision as when a Pravaz' syringe is used. The vaccine for this syringe is sent out in tubes identical with ordinary tubes, except that both extremities are closed by small caoutchouc corks. The smaller cork is to be withdrawn and the extremity of the tube introduced into the reservoir of the syringe: on removing the other cork the liquid runs into the reservoir. In all cases, whatever kind of syringe be adopted, a tube of vaccine which has once been opened must never be used on the next or following days, for in that interval the vaccine liquid may have become altered by foreign organisms. If the vaccine when sent out cannot be used for three, four, or five days, the tubes ought to be put in a cool place, if possible in a cellar, in order to prevent the possible ulterior development of foreign organisms. As a rule, and whenever it is possible, fresh vaccines ought to be used, and used on the day or the day after they are received. When it is desired to keep vaccines for a long time—one, two, or three months—it is necessary to take special and very delicate precautions. This we do in the case of all vaccines sent to foreign countries. As to any vaccine liquid remaining in the tube when the operation is finished it ought to be destroyed by plunging the tubes into boiling water.

Method of preparing the vaccine in vogue at M. Pasteur's laboratory.—A drop of blood taken with purity from an animal dead of anthrax is sown in flasks of sterlized broth, and the flasks placed in an

incubator kept at 42°-43°C. At the end of twenty-four hours a drop of culture is taken from one of these flasks and sown into another flask of pure chicken broth; this latter is placed in an incubator at 30°-35° C., whilst the original flask is again returned to the incubator at 42°-43°C. This operation is repeated every twenty-four hours, so that at the end of 15 to 20 days, we have obtained a series of more and more attenuated cultures. From among the latter day cultures, usually those of the tenth or twelfth day, a culture is chosen which makes the "premier vaccin" and another, less attenuated, ordinarily that of the 7th or 8th days makes the "deuxieme" or second "vaccin." The filaments of these vaccines are cultivated in suitable media, and the resulting spores placed in glass vessels or tubes sealed by heat, and known as seed tubes. This process is conducted in the Pasteur institute.

Manner of sowing the vaccines.—The seeds of the first and second vaccines are sent in tubes closed at both ends. The tubes are opened by cutting the superior extremity by means of a file or glass knife. The fluid in the tube is then aspirated with a pipette which has been previously sterlized by exposure to a temperature of 200° C. This fluid is sown into three little flasks of chicken broth as accurately divided into each as possible, and the flasks are then placed in an autoclave maintained at a temperature of 30°-35°C. After the expiration of three days they are removed from the autoclave and placed in a cool place at from 5° to 10°C. and shaded from the light. Each of these small flasks serves to sow large flasks of veal broth for a period of five days, so that the three are sufficient for fifteen days' seed supply for the large flasks, and hence, if a continuous supply be desired, it is necessary to repeat the process of sowing fresh small flasks every twelve days. So that by the 15th day a fresh supply of material for sowing the large flasks may be ready. To sow the large flasks from the small ones all that is necessary is to aspirate five or six drops of the material from the small flask and place it in the large one, and this is accomplished by means of a capillary tube. When sown the large flasks are again returned to the incubator, some of them being allowed to remain for three days only, whilst the remainder are kept in for a period of ten days. In this way we obtain two "vaccines," one of which, *viz.*, that kept in the incubator for three days, is called "recent vaccine," and that kept in for ten days "ancient vaccine." The "recent vaccine" is more active than the "ancient," as at the end of three days the development of the filaments is almost complete and few spores are formed. This recent vaccine it is necessary to use very soon after its manufacture, because the filaments being ærobic perish after a short time in the vaccine tubes, there being no air in them. The "ancient vaccine," on the contrary, has passed on to the stage of spore formation and contains more spores than fila-

ments, and the spores are capable of resisting for a longer period in the tubes, so that consequently, although the "ancient vaccine" is in reality more attenuated than the recent, it is capable of preserving its action for a far longer period of time. When a vaccine has to remain in tubes for a longer period than two days, we take advantage of the above peculiarities and mix the "ancient" with the "recent vaccine." It is necessary to do this, owing to the fact that if the spores only were put in in their thus attenuated form, the vaccine would be too greatly attenuated and have lost in some measure its properties. Both the first (premier) and second (deuxieme) vaccines are treated in the same way. From the flasks, vaccine tubes are filled by means of pipettes and are ready for despatch. The vaccines are distinguished by different labels; the vessels containing the first bear a red label marked "premier vaccin;" those holding the second blue, marked "deuxieme vaccin." Those containing "ancient vaccine" are marked with blue pencil on red label for the first, and with a red pencil on blue label for the second. The secret in the manufacture thus appears to consist in the manipulation of the "ancient vaccine."

Proofs of the efficacy of preventive inoculation against Anthrax.—Preventive inoculation against charbon, according to Pasteur's method, has already received during several years complete confirmation; its efficacy has been recognized in laboratory experiments and in experiments conducted upon flocks in various parts of France and other countries. Some experiments from amongst a large number were made in 1881-82, which establish the efficacy and the small risk which attend preventive inoculation.

Experiments made at Pouilly-le-Fort.—Inoculation with the first and second vaccines at an interval of 12 days, on 24 sheep, 1 goat, and 6 cows; inoculation of non-attenuated virus was carried out 14 days afterwards on the vaccinated animals, and also as a test experiment on 24 sheep, 1 goat, and 24 cows non-vaccinated. The inoculation produced severe symptoms, both local and general, in the four non-vaccinated cows, which did not, however, succumb to the disease; there was no disturbance in the vaccinated animals. The non-inoculated sheep and goats died, whilst those protected by vaccination showed no symptoms of the disease. One of the protected sheep died on the fourth day following inoculation with the strong virus, but the autopsy showed death from death of the fœtus. Seven months afterwards six vaccinated sheep were inoculated afresh with strong virus and resisted, whilst four non-vaccinated sheep inoculated in the same manner died.

Experiments at Fresne.—Ten sheep were vaccinated at two successive inoculations after 12 days' interval, and resisted inoculation with strong virus afterwards; whilst of ten non-vaccinated sheep six died as

a consequence of inoculation with the strong virus. The vaccination of 1,081 sheep and a number of large ruminants and horses by two successive inoculations were unaccompanied by any serious accident, exception the death of one sheep after the second inoculation; this might have been due to spontaneous charbon. The vaccination of ten sheep by a single inoculation with the virus No. 2 enabled them to resist the inoculation with strong virus; one non-vaccinated cow was very ill after inoculation with strong virus; whilst a vaccinated cow supported the inoculation with no ill effects.

Experiment at Rossignal.—A non-vaccinated horse died of inoculation with the strong virus, whilst a filly vaccinated at two successive inoculations in the ordinary way supported inoculations with a strong virus with no ill effects.

Experiments at Chartres and Alfort.—Sheep vaccinated are not affected by the attenuated virus in any serious way; having been inoculated twice in succession, they resist inoculation with charbonous blood taken from a sheep dead four hours of spontaneous charbon. Nineteen sheep, vaccinated at Alfort by M. Pasteur, resisted inoculation with charbonous blood, whilst of 16 non-vaccinated sheep 15 died after inoculation with blood, taken from the same charbonous sheep.

Experiments at Arteney.—Vaccination was practised in the farm of d'Herblay at Arteney near Orleans on large and small ruminants. Charbon existed amongst the flock and had claimed 69 victims in less than three months. Two hundred and fifty sheep as well as seven large ruminants received the first vaccine. In the 14 days which elapsed between the application of the first and second vaccines spontaneous charbon had destroyed 8 sheep out of the 250 vaccinated; six days after the second vaccination 1 out of the 242 sheep which had undergone it died of charbon; five non-vaccinated sheep were inoculated with strong virus and died.

Numerous vaccinations were carried out in July, August and September 1881. The mortality was ten times less in vaccinated than in non-vaccinated animals and flocks of sheep, in which the vaccinated and the non-vaccinated animals lived under the same conditions. The mortality in vaccinated cows was 14 times less than in non-vaccinated (10 died out of 888 non-vaccinated and 1 only out of 1,254 vaccinated). In flocks in which charbon was present in a severe form and a portion only of which had been vaccinated, a mortality of 1 in 20 non-vaccinated occurred whilst amongst the vaccinated none succumbed.

Experiments at Toulouse.—Nine sheep were protected in three inoculations and afterwards resisted strong virus; 2 sheep protected by one inoculation also resisted strong virus. Of five non-vaccinated sheep four died and the other was rendered very ill by the inoculation with the strong virus. The same results were obtained at Lyon.

Experiments at Nevers.—Two mares, 6 oxen and 10 sheep vaccinated in the ordinary manner resisted inoculation with the strong virus, whilst 1 mare acting as a control animal died as the result of inoculation with the strong virus; also 1 out of 4 control oxen and 7 out of 7 control sheep died; 1 control ox died of septicæmia and 1 sheep from inoculation with the second vaccine.

Experiments at Mer.—Ten cows and 20 sheep protected by two successive inoculations and I sheep which had been inoculated with the second vaccine only, evinced no ill effects from the vaccination. They were inoculated with strong virus. The cows resisted and seven of the vaccinated sheep died; 10 non-vaccinated sheep died from the strong virus and 5 cows used as control animals were rendered very ill. The death of the 7 vaccinated sheep was due to the fact that the second vaccination had been carried out with the first vaccine. In a new experiment made at Mer, 15 sheep were vaccinated with two different vaccines and afterwards resisted the strong virus, whilst the control animals, numbering 15, died of charbon after inoculation with the strong virus.

Experiments at Montpelior.—Six sheep vaccinated in the usual way resisted the strong virus, whilst five non-vaccinated sheep died.

Experiments at Bordeaux.—Vaccination having been practised upon 188 sheep and 17 cattle, œdema was observed in the cattle after the second inoculation; abortion of a cow and death of two vaccinated sheep occurred; 8 sheep, 1 ram and 1 calf non-vaccinated, having been inoculated with strong virus, as well as 18 sheep and 2 cows vaccinated, including the one which had aborted, all the non-vaccinated sheep and the cow, as well as 1 out of the 18 sheep vaccinated died, whilst slight disturbance only occurred in the vaccinated cattle.

Experiments at Angouleme.—Twelve vaccinated sheep resisted the strong virus, whilst 7 control animals succumbed.

Experiments at Clermont Ferrand.—Five vaccinated sheep resisted the strong virus, whilst 2 control animals died; one vaccinated ox resisted and one control died. Similar favourable results from Pasteur's method of inoculation were observed at Nimes, Salon, Nogent-Sur-Marne and in other localities.

Experiments in Austro-Hungary.—In the experiments made by Thullier at Buda Pesth, the inocuity and efficacy of vaccination were recognized both as regards sheep and oxen. At Kapuvar 14 vaccinated oxen resisted inoculation with the strong virus, whilst in 6 control animals 3 died and 3 were rendered very ill: in sheep vaccinated, accidents occurred which were due to the impurity of the vaccine, to the weakness and too great receptivity of the animals, and to the too great activity of the vaccines.

Experiments in Germany.—In the experiments made by Thullier 25 sheep and 6 oxen were vaccinated; 3 sheep died from the second vaccine; the 22 sheep remaining and the 6 oxen resisted inoculation with the strong virus, whilst 24 out of 25 sheep used as control animals succumbed, as well as 3 control oxen out of 6, the three others being seriously ill. A fresh experiment was made with a weaker vaccine, 128 sheep and 123 lambs having been vaccinated whilst 128 sheep with 123 lambs served as control animals; one sheep died after the second inoculation; 12 vaccinated sheep and 12 lambs were inoculated with strong culture virus, or with charbonous blood, and resisted, with the exception of two lambs which succumbed; 12 control animals which received inoculation with strong virus all died. After these experiments having been made in 1882 at Packisch and Borschutz the "Gazette National" of Berlin published new results which showed that bovines and ovines succumbed in less numbers when inoculated, on lands where anthrax was prevalent. In the district of Packisch the mortality from charbon had decreased considerably in oxen and sheep, thanks to vaccination.

Experiments in Italy.—Perroncito, having inoculated 8 sheep, 3 goats, and 2 oxen at twelve days' interval in the ordinary way, observed slight fever after the second vaccination; he made a third vaccination with a little stronger second vaccine twelve days after, and the animals evinced no disturbance. Six of the vaccinated sheep, 2 goats, and 2 oxen were then inoculated with strong virus and resisted, whilst of 8 animals used as control, perished from anthrax and the eighth recovered after having been very ill; 2 oxen which had not been protected, inoculated with strong virus, perished from anthrax.

First Experiment at the School of Turin (Bassi).—Three horses, 2 oxen, 1 ram, 5 sheep previously vaccinated were inoculated, together with 6 sheep, 2 oxen, and 2 horses non-vaccinated; the inoculation was made with the blood of a sheep which had been dead of charbon for 24 hours, and it caused the death, probably from septicæmia, of all the non-vaccinated animals but one horse and one ox; it also destroyed 1 horse, 1 goat, and 5 sheep, which had been vaccinated, that is to say, all excepting 2 horses and 2 oxen.

Second Experiment at the Turin School (Bassi).—Thirteen sheep, 2 horses, and 3 oxen were vaccinated and then inoculated together with control animals, 6 sheep, 2 oxen and one horse, with a non-attenuated Pasteur cultivation,—4 sheep and 2 oxen non-vaccinated. All the vaccinated animals resisted, and all the control animals, excepting 1 ox which was very ill and died. Charbonous blood taken from a sheep two or three hours before death was inoculated to 600 ovines, 2 bovines, and 1 horse which had all been vaccinated; and into 4 sheep, 2 oxen, and 2 horses

non-vaccinated ; two vaccinated sheep died, and all the non-vaccinated animals with the exception of 1 ox.

At the Congress of Geneva in 1882.—Dr. Sormani, member of the Commission, charged with the surveillance of the vaccinations for charbon at Milan and President of the Commission which had carried them out at Pavia, declared that at the commencement, the experiments conducted in Italy, at Milan, Turin, Bolognia, Pisa and Pavia all had not given favorable results. In some cases the animals died from the vaccination, in others the vaccinated animals died of the test inoculation made with strong virus. This was due to the fact that vaccine prepared for use in oxen had been employed to vaccinate animals more susceptible. Animals which had had no fever after the second inoculation had sometimes succumbed to the inoculation with strong virus, whence the necessity of inoculating a third time animals not having had fever, or augmentation of the temperature after the second vaccination ; the accidents which occurred in the vaccinated in the first experiments at the Turin School did not recur in the experiments made by Perroncito, nor in those of Sormani of Pavia, nor in experiments on bovines. Dr. Sormani concludes as follows : " I can then declare that the experiments with vaccination for charbon have been successful, having had a truly scientific control, being carried out with the most rigorous precautions, without blind enthusiasm or pre-conceived, or wrong ideas. Vaccination for charbon forms one of the greatest glories of scientific France, and of M. Pasteur, her immortal son."

Experiments in Belgium.—The efficacy of Pasteur's method of vaccination was recognized at Herve in bovine and ovine animals.

In Switzerland.—M. Guillebeau of Berne recognized the fact that two preventive inoculations preserved animals against inoculations with the strong virus when it is inoculated in small quantity, but not when in large quantity.

In Spain the efficacy of vaccination has been equally recognized. It is also acknowledged in Holland, Algeria, Russia, England, Australia, etc.

Almost the whole of the experiments made then have from the first been followed by very encouraging results, despite the fact that strong virus has been inoculated in order to demonstrate the immunity conferred. For sheep resist the ingestion of charbonous germs much better than their inoculation, doubtless for the reason that by the latter method larger numbers are introduced, so that vaccinated animals resist natural contagion better than control inoculations as statistics have clearly demonstrated.

In 1881, in flocks of which a moiety had been vaccinated and the rest had not, all the animals living together, the mortality from charbon amongst the sheep and cattle which had been vaccinated was much less

than amongst the non-vaccinated. In fact, in certain flocks in which charbon had been severe, it was observed that the mortality had been *nil*, or nearly so in the vaccinated, whilst it had remained high amongst the non-vaccinated. In 1882 vaccination considerably diminished the mortality from charbon in flocks and herds. These results were improved upon during the following years; so that the mortality from charbon which had been 8 to 10 per cent. amongst sheep in flocks exposed to the disease, has fallen to 1 per cent. after vaccination; it has equally lowered the mortality amongst bovines.

Vaccination against charbon in Hungary.—M. Hutyra, of Buda Pesth, notes that during the year 1890, 939 horses, 9,356 bovines and 78,770 sheep were vaccinated against charbon. The losses from the operation owing to the ulterior evolution of charbon were—

3 horses or 0·31 per cent.
36 cattle or 0·37 „
1,571 sheep or 2·00 „

From observations made, it appears that neither the race nor age of the subjects inoculated had any influence upon the termination of the operation (Journal de med vet and de zoot, January 1892).

Accidents consecutive to vaccination.—At the commencement, vaccination sometimes gave rise to more or less grave accidents. We witnessed the appearance of œdema, tumefaction more or less extensive in oxen and solipeds, following the second inoculation, and cases of death occurred amongst sheep as well as large ruminants. We found cases in which bovines which had been inoculated for two or three months only, succumbed to anthrax. It was recognised that the vaccines weakened gradually with age. The first vaccine became too feeble and did not always preserve the animal against the second, which destroyed it occasionally. In other cases both the vaccines became so enfeebled that they did not give immunity from charbon, which carried off animals even during the first two or three months following vaccination. Since that time—thanks to a more profound knowledge of the condition of attenuation and of the mode of preservation of the attenuated virus—we have succeeded better in avoiding the accidents which occurred at the commencement. Nevertheless, we must not deny the fact that vaccination is attended with certain risks to animals, which cannot be avoided completely, for they may be influenced not only by the quality of the vaccine, but by the susceptibility of individuals, breeds, species, and defects in the operation. The accidents which have been observed should without being exaggerated be made known to all veterinarians, so that they may not be led to promise invariably excellent results to persons seeking to have their animals protected, but may be able to communicate

to them more or less correctly the risks run. Vaccination has resulted in death following either the first or the second vaccine. At other times grave accidents, such as dangerous illness, œdemata, engorgements, etc., have followed it; at other times, it has not conferred immunity at all, or only for a very short time, so that we may see sheep and cattle die after the first and second inoculation. Animals subjected to the two inoculations may contract fatal charbon during the first two or three months following inoculation. We may observe very extensive œdema, and in some cases death following the second inoculation in the horse. Moreover, it is a generally admitted fact that vaccination for charbon is more dangerous in the horse than in ovines or bovines; that it very frequently gives rise to very serious œdemata and may cause severe symptoms, even death, and that it is not advisable to have recourse to it in these animals excepting under exceptional and urgent circumstances such as during an epizooty. It has also been found by Biot, Lamare, Brillant, and Duclaux, that the bad quality of food and management to which animals are submitted exercises a great influence on the gravity of the accidents determined by inoculation. M. Biot, after inoculating 125 cows with the premier vaccine, saw the following accidents appear: all the beasts became ill; 25 or 30 were very ill, had much fever and considerable œdema; 30 to 35 were attacked by charbonous fever of a severe character with enormous œdema, and some with secondary œdema and colic. These accidents were explained by the bad quality of the food. A similar observation has been made by Duclaux in Auvergne (Cantal). Local and general accidents more or less intense according to the quality of the food, are produced, more intense in bovines and sheep in barren localities, where vaccination destroys $\frac{1}{4}$ or $\frac{1}{6}$ of the sheep, whilst the same virus only destroys $\frac{1}{50}$ well nourished sheep.

We have also seen engorgements, with general symptoms more or less alarming, appear in large ruminants following the inoculation with the second vaccine. The inoculation of sheep in an advanced stage of gestation sometimes gives rise to abortion. Young lambs may also be rendered very ill, etc. In some cases it has happened that inoculation has given rise to serious losses, but this is fortunately of rare occurrence. Koch has noted the following points:—(1) that if the vaccines are too strong, they may cause fatal charbon; (2) if they are too feeble they do not protect; (3) that they do not confer immunity without rendering the animal seriously ill, and that they are consequently dangerous; (4) that vaccination may serve to disseminate charbon by rendering animals severely ill from that disease, during which illness they are capable of infecting others; (5) that the immunity conferred is insufficient to preserve with certainty from natural infection; (6) that the immunity conferred is of too short duration, etc.

Indications for vaccination. Duration of the immunity. Precautions to be observed.—Despite everything, there is reason to consider that vaccination is capable of giving favourable results. The accidents to which it gives rise are in reality of little importance. Vaccinated animals ordinarily become sufficiently refractory to resist natural infection and even experimental inoculation. It has, however, been found that virus sent to a distance is not very easy to preserve, and that in order to obtain the best results with the least risk, it should be employed as soon after its preparation as possible. In order that this may be the more easily done, laboratories have been founded in various countries distant from France, where the attenuated virus can be prepared; the efficacy of vaccination has been recgonized and attested in all parts; it is recognized in Belgium, Spain, Italy, Switzerland, Germany, Austria, Russia, America, Australia, etc., as in France, that it confers immunity, and that it may be advantageously employed to preserve animals of the ovine and bovine species from natural charbon After having been tested, vaccination has been practised in the above countries. For example, in Italy, in order to render it popular, it has been decided to deliver the vaccine free to veterinary surgeons.

Knowing the causes which may tend to aggravate the dangers of inoculation, we should always in practice do our best to avoid or at least to lessen them. We should not vaccinate horses, as we have already mentioned, unless in exceptional cases. We should employ vaccines as fresh as possible, and take care to keep them cool, sheltered from the air and in a dark place until wanted for use; some have employed glycerine to preserve it (Cienkowsky), but it is better to proceed as we have already stated. We should always exercise the greatest care, and use instruments perfectly clean, so as to avoid all mixture of impurity or foreign germs with the virus. We should always keep in view that the breed, age, condition of individuals, their food, etc., may exercise a great influence on the operation; we should therefore, before inoculating on a large scale in countries where vaccination has not been practised, sound the ground by some preliminary trials on a small number of subjects of various ages, breeds, etc. We should vaccinate sheep only when not in lamb, or at an early period of gestation. We should not vaccinate them when in an advanced stage, unless under urgent circumstances, as when the malady is severe in the flocks. Lambs should not be vaccinated unless there is reasonable fear of their contracting spontaneous charbon; although it is understood that they may receive similar doses to adults, we may content ourselves with using smaller doses. We should choose a time to inoculate the large ruminants when they have not to work, and for cows a time when they are not in full milk, because vaccination causing fever, diminishes the

yield of milk, and may, if charbon should accidentally follow the operation, render the milk dangerous. Moreover, the milk of vaccinated cows should be rejected for fifteen days after the second inoculation. It is necessary to diminish the dose for individuals and races endowed with a more marked susceptibility, and also for animals placed under bad hygienic conditions, badly fed and housed, etc.

We should vaccinate for preference in the spring time, when the disease is not so prevalent, so as to avoid the chance of increasing the risk of vaccination being accompanied by the spontaneous disease, and also for the reason that animals freshly vaccinated have greater resistance to the spontaneous disease at the end of spring and during summer when it is most generally prevalent. We should inoculate sheep at the inside of the thigh, as has been already pointed out. With regard to the larger ruminants, we may inoculate them behind the shoulder, on the flank, or on the tail and ear: we should pierce the skin with a strong needle and the injection should be made into the subcutaneous tissue. The œdematous swellings which may result should be left alone, no treatment being permitted when they are not very extensive and are unaccompanied by any alarming general disturbance. If otherwise, we should abstain from opening them, but we may inject into them phenicated water at 2 per cent., or iodised water. To recapitulate then, the evidence which is available to us has for the most part gone to prove, despite the denial of some, that vaccination has considerably decreased the mortality in flocks exposed to the ravages of charbon, but it is on the other hand averred that it is not entirely free from some dangers; there is reason then not to press its adoption, excepting in countries where the loses from anthrax far exceed the losses consequent on vaccination. The vaccination of bovines causing only insignificant losses and diminishing the mortality from charbon to a considerable extent, is indicated in all countries where the mortality from charbon exceeds 1 or 2 per cent. The vaccination of sheep also, although it may occasion more sensible losses, nevertheless presents notable advantages in all countries where the mortality from charbon exceeds 3 or 4 per cent. It should not, on the other hand, be practised imprudently; it should not be adopted in a country where charbon does not exist, nor even in those where it is of rare occurrence. With regard to the duration of the immunity conferred by Pasteur's vaccine, it has not been decided in an absolute manner, nor has the question of the hereditary transmission of immunity been well made out. Lambs born of Algerian mothers inoculated during gestation, should be refractory to the disease according to M. Chauveau; it cannot be the same in the case of lambs born of French mothers during gestation (Rossignol) or even before (Toussaint). But contradictory results have been obtained by the Commission of the

"Société centrale de médicine vétérinaire" on the question; the Société d'Agriculture de Melun have noted a lamb born of a vaccinated ewe die from inoculated charbon, and again it is generally recognized that lambs born of vaccinated ewes do not generally enjoy immunity (Chamberland).

The duration of the immunity conferred by vaccination varies much according to the strength of the virus inoculated, and according to the degree of receptivity of the animals; it may vary from some months to a year or more. It has been observed to last for 2, 3, 4, 5, 6, 7, 8, 9, 10 months, so it may even last 11 or 13 months, etc. Sheep may still remain protected in a proportion of 60 per cent. after the end of a year (Chamberland). However this may be, it may occur, as has been observed, that immunity does not last more than 2, 3, or 4 months, etc. The duration of the immunity seems, all other things being equal, to be in direct proportion to the intensity of the vaccine, and of the feebleness of the receptivity of the animal. To obtain lasting immunity, it is necessary then to employ the strongest safe vaccine for animals endowed with the greatest receptivity, but the dangers of vaccination are thus increased. In all cases the degrees and the probable duration of immunity may be gauged by the amount of re-action which follows vaccination.

Cases have been observed of the duration of immunity for two or three years; but it is presumed either that the immunity had been conferred by the action of strong virus, or that it had been afterwards renewed by natural infection, which, although incapable of rendering the animal seriously ill, had contributed to perpetuate their refractory state. It has, however, been decided that in order to perpetuate the benefits of vaccination in localities where charbon is prevalent, it is necessary to inoculate yearly.

Chauveau's method of vaccination.—Vaccination, with the virus attenuated in the air, or in compressed oxygen, according to M. Chauveau's method, has been practised with success in France, Chili and Switzerland. It has given good results in the sheep, large ruminants and even in the horse. The mode of operating is the same as by Pasteur's method. From experiments made previous to and those of 1888 before the Society of Agriculture, Melun, the following conclusions were arrived at: vaccine prepared by the compressed oxygen method is endowed with great fixity; it may preserve its efficiency for several months and can be transported to great distances; it also confers complete immunity by a single inoculation; and it does not give rise to the same amount of œdema in bovines and solipeds, as Pasteur's vaccine does; it enables us to confer immunity twelve or fifteen days sooner than Pasteur's vaccine does. It may be added that multiple and successive inoculations with the same vaccine confer a

more complete immunity. We may remark that immunity may be conferred by a single inoculation with Pasteur's lymph by choosing a strength intermediate between that of the first and second vaccines generally used. For the rest, the majority of the considerations mentioned *à propos* of vaccination Pasteur apply to M. Chauveau's method; the same rules for the mode of operating, the same precautions to be observed, the same indications for the operation, etc., etc.

Means of conferring immunity against charbon.—The "1st vaccine," Pasteur, produces little effect upon the rabbit; it does not injure the adult guinea-pig, but it kills mice and very young guinea-pigs.

The "2nd vaccine," Pasteur, inoculated to the guinea-pig always kills it and destroys also a certain percentage of rabbits. The rabbits which resist its action may be vaccinated. When we have first inoculated the "1st vaccine" a larger percentage of rabbits resist the second, but some still succumb; inoculation with the 1st vaccine does not always enable them to bear the action of the second. We are able, however, by proceeding as M. Feltz has done at Nancy in 1882, to confer immunity on the rabbit by subjecting it to three or four successive inoculations with virus increasing virulence and intermediary between those of "1st vaccine" and that of "the 2nd vaccine." MM. Roux and Chamberland have succeeded in enabling the rabbit to support the "2nd vaccine" by the intravenous injection of 4 cc of a culture of "1st vaccine," repeating it after two or three days' interval; a week afterwards, the rabbit so treated bears the injection of O.cc 25 of "2nd vaccine" under the skin and acquires immunity against charbon. The bacilli of the 1st vaccine injected in such large numbers into the blood, disappear rapidly, accumulating in the spleen, but do not multiply, and become rapidly destroyed, so that it is uncertain whether the immunity from the effects of the 2nd vaccine is due to the products of the culture or the products of the destruction of the bacilli. According to M. G. Colin inoculations of small quantities of charbon blood, repeated several times, may confer immunity upon the ass, dog and horse, which may after a certain number, support the action of an enormus injection. But all individuals do not resist these inoculations with strong virus, and hence this method of proceeding does not merit further consideration.

It appears that immunity against charbon has been produced occasionally by transfusing the blood of the dog into the inoculated animals (Rondeau) or the blood of birds; the serum contains bactericidal substances capable of preserving the organism against the invasion of the bacilli; but these substances which are soluble in water, and not in alcohol, may be destroyed by acids, by the digestive organs, and by a temperature of 45° C.

On the other hand, although certain experimentalists have main-

tained the contrary, it appears to have been demonstrated by others that the serum of either dog's or frog's blood is not prophylactic. It has, however, been found that the serum of rat's blood is endowed with bactericidal properties "in vitro," and that if it be mixed with the bacilli injected into the rat, it hinders or retards the development of charbon according as the germs are in the filamentous or the spore stage. The protection conferred by rat's serum has been made the subject of investigation by Mr. Hankin, who states that it is due to particular substances which he has called albumoses.

It appears satisfactorily demonstrated to-day that we may create immunity by means of chemical substances, and more especially with the soluble products resulting from the life of the microbes. Thus, according to Kasch, animals which have taken a sufficient quantity of sublimate will not be susceptible to charbon: Behring says that it is the same with those to which salts of silver have been given. According to Woolridge we may obtain immunity for the rabbit by inoculating him with a liquid taken from the bacilli, the liquid obtained by filtration of a culture developed in an alkaline solution of an albuminoid substance obtained from the thymus or the testicle.

MM. Chamberland and Roux have demonstrated, as have also Chauveau and Woolridge, that the bacillus secretes a material which possesses vaccinal properties. The following is a résumé of their researches on this subject: Toussaint had attributed the immunity to the action of chemical substances, although in reality he was vaccinating by his method with the attenuated microbe; MM. Chamberland and Roux have found that it is possible to confer immunity on sheep by the subcutaneous injection of charbonous blood deprived of living bacilli by heating for 40 minutes at 55·5° C.; there exist, then, in the blood of an animal dead of charbon, chemical substances capable of conferring immunity on sheep into which a sufficient quantity is injected. But long exposure to heat, or exposure at too elevated a degree, changes these substances at the same time as it kills the microbes. Thus, charbonous blood heated for ten minutes at 100° or 115° C. loses its vaccinal properties. It is necessary to employ a less intense heat. In reality sheep vaccinted with decoctions of blood or of the spleen have not resisted inoculation with the strong virus, although they have been destroyed less quickly. One hour and a half's exposure to a temperature of 55° C. is not sufficient to destroy all the bacilli in the blood; thus this procedure is full of uncertainty, and it is better to have recourse to some other to combat the microbes. M. Pasteur has adopted the following method for the production of immunity in rabbits; he takes blood from an animal about to die, closes it in a tube which is quite filled, and then seals the ends; under these conditions the bacillus does not

multiply nor form spores, but is destroyed in 12 days at + 45° C., whilst it lives for more than a month below + 17° C. MM. Chamberland and Roux have adopted the following method: aspirate charbonous blood into two tubes, fill them completely, close the ends with the lamp, and then plunge them for an hour into a bath of water kept at 58° C.; then take them out and heat them a second time for an hour; repeat this operation for five consecutive days; the blood so heated has never given cultures when sown in suitable media, but its vaccinal properties are weakened, and hence it is necessary to employ it in large quantities. The liquid which flows from the clot from the tube is used for inoculating sheep. Sheep which have received charbonous blood which has been previously heated at 58° C. in sufficient quantity inoculated into the cellular tissue, have resisted inoculation with the strong virus; however, they have been rendered seriously ill by the inoculation of control, which demonstrates that immunity conferred by means of heated blood is less reliable than that brought about by the attenuated virus. The amount of blood required to produce immunity varies in different individuals; some have been protected by 8cc, and others have not, even after the injection of 16cc to 32cc. Sheep show themselves more refractory when they have received larger injections of heated blood. The immunity conferred by these methods is not a lasting one, its duration being only for a few days; after a lapse of 24 days it has been found that two out of every three animals have lost the protection. Blood, prepared in the above manner and filtered, was injected by MM. Chamberland and Roux into the vein of a sheep in large doses. The sheep bore the injection of large quantities without any sign of intoxication, but the immunity conferred was very feeble, even less than that following subcutaneous inoculation, a fact which might lead us to the conclusion that the vaccinal substances were either eliminated by the kidneys or some other organ, or else partially destroyed by oxidation. The injection of charbonous blood previously filtered through porcelain has not succeeded in giving immunity to sheep when inoculated into the veins. Might it not, from a consideration of these facts, be inferred that the vaccinal material is a diastase, and hence its alteration by heat and its not passing through a porcelain filter.

Although charbon does not readily lend itself to a decisive demonstration of vaccination by chemical substances on account of the ease with which the vaccinal material is changed by heat and re-agents, still it remains certain that immunity can be conferred against charbon, by means of blood deprived of bacilli, and it is the modified method of Toussaint, by heating, which best shows it. Koch and Chamberland consider that it is preferable to kill the microbes by the use of essences,

which have no action on the albuminoids nor the diastase. Essence of mustard is recommended by Roux and Chamberland. Charbonous blood and the fresh pulp of a charbonous spleen treated with essence of mustard and evaporated *in vacuo* to eliminate the essence when its action is completed, gives a residue which contains fixed microbian products and which confers immunity on the rabbit with smaller doses than the same material filtered or heated to 58° C. We may also obtain similar results by the use of Eucalyptus (De Christmas). However, we must not lose sight of the facts that the bacillus which produces vaccinal substances in the organism and in the blood, produces but little in cultivations, and that the immunity conferred by these substances is of much shorter duration than that resulting from the action of living vaccine.

G. H. Roger has performed some new experiments which have equally demonstrated that the bacillus produces a vaccinal material. Cultivations heated for from 5 to 10 minutes at a temperature of 110° to 115° C. were then injected into the veins of rabbits, either before or after filtration; it appeared that it contained a vaccinal material which remained in the bacilli, and which when deposited in the tissues might be dissolved and re-absorbed in order to produce its effect. Lastly, M. Chauveau's experiments have demonstrated that cultures destitute of all virulence confer immunity, which clearly proves the existence of a vaccinal substance.

Mechanism of conferred immunity.—Conferred, like natural immunity, is due to the bactericidal properties of the humors and to the phagocytic power of the cells.

Vaccination for charbon, that is to say, the production or creation of immunity by means of attenuated virus, is an effect of the life and of the multiplication of vaccines in the animal body by which the economy is in some way rendered able to resist the action of bacteria and to support their poisons (Gamaléia). If we seek to enquire more intimately into the mechanism of vaccination, we find ourselves a little embarrased, as the facts connected with the question are sometimes contradictory. The following, however, is a brief résumé of the results of various researches.

MM. Chamberland and Roux, in vaccinating the rabbit, have observed that the microbes of the first vaccine inocculated subcutaneously are rapidly destroyed at the seat of inoculation, and that those which have been injected into the veins promptly disappear from the blood to congregate and preserve themselves in the spleen for a longer time. Bitter, experimenting on the sheep, has noticed that the vaccinal microbes are destroyed at the seat of inoculation, and that they do not disseminate themselves generally. Gamaléia considers that the bactericidal state of the humors of vaccinated subjects is not due to the cells

which they contain. He attributes the destruction of microbes, partly to the action of phagocytes, and partly to the bactericidal action of the humors. He has observed that the bacillus inoculated into a well vaccinated sheep provokes œdema without diapedesis, and that it is destroyed for the most part at the seat of inoculation where the œdema exists. He believes that immunity is not conferred unless vaccinal fever is produced by the inoculations, during which the microbes or their secretions enter the blood and organs. Perroncito thinks that the most resistant virus is destroyed rapidly in the tissues of the vaccinated animal. Wissokovicz believes, as do also Chamberland and Roux, that vaccination is the result of the action of soluble products of the organism; he believes that the germs of vaccine material do not propagate in the body, and that they are destroyed, not by the leucocytes, but by the fixed cells. Lubarsch has not observed the general dissemination of the bacilli in the body in two sheep vaccinated by the subcutaneous inoculation.

Is immunity due to a general dissemination of the vaccine in the body? It appears, according to certain works, that the vaccines do not propagate, and that vaccination is due to products manufactured on the spot by the microbes or introduced with them. In studying this question, Metchnikoff has arrived at the conclusions which follow: "The bacilli of the charbon vaccine produce their effect by cultivating at the point of inoculation without necessarily becoming disseminated throughout the organism; only exceptionally do they penetrate, and then only in very small quantity into the organs, so that this penetration does not play an important part in the protection of the animal. The humors of vaccinated animals, deprived of their cells do not contain products harmful to the development of the bacilli. The destruction of the bacilli takes place at the point of inoculation, and is brought about by the activity of micro and macro-phagocytes, especially the leucocytes. Vaccination doubtless consists in the cellular elements becoming accustomed to the toxic products of the bacilli. This latter conclusion is based on the fact that in the vaccinated animal, vaccines and virus develop normally in the aqueous humor deprived of cells, whilst they are destroyed in the organism where they are accessible to the action of the cells.

He finds that in sheep, the two vaccines provoke local phenomena and produce their effects by reason of their local culture, that the bacilli do not multiply in the organism, but are destroyed by the cellular elements and more particularly the leucocytes, that the results of the experiments made on rabbits accord in general with those which occur in the sheep, that the microbes of the first vaccine introduced into the blood do not multiply, that those of the second vaccine introduced under the skin rarely penetrate outside of the seat of inoculation.

When we inoculate the bacillus into a non-vaccinated and into a

vaccinated rabbit and a dog, we observe that in the non-protected rabbit the bacilli multiply, determine an œdema, tumefaction of the ganglia, general disturbance and death; in the vaccinated rabbit and the dog the bacillus also multiplies at the seat of inoculation, determines an œdema richer in leucocytes and is destroyed without becoming generally disseminated.

Diagnosis.—It is of the highest importance to be able to diagnose cases of charbon with promptitude and certainty from the triple point of view of treatment, prophylaxy and sanitary police, hygiene and public health. It is in reality by appropriate and early measures that we must endeavour to avoid the dangers of the disease, limit its ravages, hinder its transmission to animals and man, facilitate recovery at least in man and some animals. For the promptitude of an enlightened and appropriate intervention depends upon the celerity and certainty of the diagnosis.

The diagnosis of charbon is sometimes surrounded by serious difficulties, more particularly when we have to deal with isolated cases, and when we have only the assistance of symptoms and macroscopic lesions which are, in the majority of cases, little pathognomonic, and which besides differ according to the species of animals and to individual peculiarities. It becomes possible, and even relatively easy, when we know the mode of appearance and the ensemble of conditions which has governed the apparition of the disease, its course, evolution, symptoms, termination and lesions; when we are aware that similar cases have already occurred during the year or in preceding years; when we know that soiled fields and pastures are in the vicinity, and that the animals have frequented places where charbonous cadavers have been previously interred, etc., etc.

It is therefore indicated to hold a veritable inquest when we suspect the existence of charbon; to gain as good a knowledge as may be of the previous history of the case, and to adduce all the evidence which may be forthcoming to lead us to a correct diagnosis.

The diseases with which charbon may be confounded vary with the species of animal; hog cholera, pneumo-enteritis in the pig; typhoid fever, gastro-enteritis, etc., in the horse, etc., etc. But it would be superfluous to enumerate them here and to draw parallels between them and charbon, when we have certain means of diagnosis at our disposition by which the latter disease may be recognized. In short, by bacteriological examination, inoculation, and culture any one may at the present day dismiss uncertainty and establish an absolutely certain diagnosis when these means are properly and opportunely employed before the transformation of the rods and the disappearance of virulence.

Microbiological examination at a suitable degree of magnification, carried out according to the precited rules, enables us to make an

absolutely certain diagnosis, when we are enabled to find in the blood of the sick animal, in the ganglia, spleen, liver, kidneys, etc., of the cadaver, or in the meat the existence of immobile rods, straight or bent, cylindrical, in one, two or three segments, etc., when the blood globules are agglutined in irregular masses, separated by spaces in which bacilli are present, etc.

We have already seen the various means of proceeding in the bacteriological examination, with or without a clearing re-agent, such as acetic acid or formic acid, with or without staining. When we have to deal with an animal still living, we may make preparations of blood, or with the product of œdema, etc., obtained by a puncture, and also with the blood of hæmorrhages, etc., we may examine drops of blood or sanguinolent material, which may be colored between cover-glass and slide or dried, cleared, and then colored either on cover-glasses or slides. Sometimes when the disease is not sufficiently advanced, bacteriological examination does not give a certain diagnosis; the blood obtained by punctures or by bleeding may not contain any rods, either because the bacilli have not invaded the mass of the blood or because they are so few in it that they pass unperceived on account of their small number, and the small amount of material examined. In such cases it is necessary to make successive and repeated examinations. When we can perceive no bacilli, we must not base our diagnosis on the crenated or the starred condition of the blood corpuscles which may be absent in charbon and present in other diseases.

When we have to decide by a *post-mortem* examination the thing is easier, because the bacilli are more numerous, and consequently more easily discovered; and because, in addition to the blood, we are able to examine the product of the lesions and the pulp of organs reputed richest in bacilli. We make preparations of the blood, exudation, hæmorrhages, spots, ecchymoses, the products flowing from the cut surfaces of organs, as the spleen, ganglia, lung, liver, kidneys, etc.; or on the mucous membrances of the stomach, intestines, etc. We can proceed with the examination on the spot and, if a first examination is absolutely significant, it will be sufficient, whilst if it be negative, we may make others of the various organs and tissue, nervous centres and the like, when we have reason to believe that there has been special localization of the disease.

When the examination cannot be made on the spot, or when unable to make it later, the material should be sent to a competent person for examination, and special precautions have to be taken. We may proceed in various ways: we may make cover-glass preparations which may be examined later on, or sent away for the purpose; we may collect blood in a vessel or in capillary tubes, or in sterilized glass pipettes, filling the

glasses and taking care to prevent the blood being soiled with other matters or germs, sealing the ends of tubes or pipettes with the lamp, when they may be kept or sent for examination, serving for preparations, cultures, or inoculation; we can also enclose a portion of a vessel between two ligatures for the same purpose: we may take blood clots, fragments of organs, liver, spleen, kidneys, etc., and even place them in glycerine, alcohol, etc., and send to the persons charged with their examination, etc., etc. However, we must never forget to make these preparations, as soon as possible after death; the autopsy should be performed as soon as possible after death, as charbonous cadavers rapidly putrefy, and in carcasses undergoing putrefaction more or less advanced the bacteria may be found mixed with putrefactive septic germs, or they may be destroyed, transformed into spores in products where they have been in contact with the air, and bacteriological examination practised under these conditions gives an uncertain result.

The diagnosis of charbon becomes particularly important in the inspection of meat destined for the alimentation of man. The sanitary law forbidding the consumption of charbonous meat is a very just one, since it is attended with double dangers, being dangerous to manipulate and liable to transmit malignant pustule to man; dangerous when ingested, for when insufficiently or even passably cooked, it may cause charbon if spores have been formed in it. Cases of transmission to man by the manipulation of cadavers, meat, or charbonous debris are not rare. On the other hand, it is averred that the juice of charbonous meat incompletely cooked is inoculable (Boutet), and that charbon may result from eating such. Internal charbon without external manifestation has been observed occasionally in men who have ingested charbonous meat. Lorenz, in August 1884, observed three cases of death in persons who had eaten the incompletely cooked meat of a charbonous cow at Lauterbourg. At Naschevo, near Wreschen, more than twenty persons fell ill after eating the meat of a charbonous cow.

Treatment curative.—One fact has been evolved from a number of experiments which have been made regarding the vitality of the virus of this disease, which may serve as a basis for the plan of campaign, and that is that certain drugs, notably corrosive sublimate, 1 per mille, suffices to kill the spores by simply wetting them with it. These tests were however made by simply inoculating them into nutrient media, which have been treated with the antiseptic. Klien has found that if, in place of inoculating into a nutrient medium of this nature, the spores are introduced into the circulating blood of an animal, it requires much longer exposure and much stronger solutions to hinder the development of spores into bacilli, and prevent the production of anthrax.

We know, however, that anthrax bacilli, as opposed to the spores are

much more easily killed, they being destroyed by two minutes' exposure to a one per cent. solution of carbolic acid in water, whilst the spores remain alive under similar circumstances for a week or longer.

Taking these facts into consideration, it would appear to be futile to attempt to destroy the spores of the disease which have been introduced into the system of an animal, but it should not, I think, be presumed that nothing can be done to tide the animal over an attack of a moderate intensity. Taking advantage of our knowledge of the peculiarities of the different forms of the organism, we may perhaps accomplish something towards recovery. We know that spores are not developed in the organism of an animal suffering from the disease, but only rods. If, therefore, we are able to so interfere with the rods as to prevent their development and functions, we can overcome the disease, for we have no second crop of spores to deal with. If we cannot introduce those agents which from experiment have been found to have a lethal action upon the spores in sufficient quantity, we can at least endeavour by their administration to somewhat impede their development into bacilli, and to exercise such action upon the bacilli when formed as either to destroy them or at all events so interfere with their ordinary functions, development and growth as to prevent their inducing a mortal form of the malady; but in order to bring about this much to be desired result, it is necessary that we may have the earliest possible intimation of an impending attack, and I may here say that in the severer forms of the disease these measures are of little avail. It is only during the course of an outbreak in a regiment or battery, for instance, that we have the means and opportunities of applying the principles set forth. Under the circumstances attendant on outbreaks of this disease in regiments, etc., we are able by watchfulness and careful thermometric observations to decide by logical inference whether it be advisable to subject an animal to antiseptic treatment before the appearance of diagnostic symptoms of anthrax, and in certain cases I am of opinion that such a proceeding is perfectly justifiable. No harm, or an infinite small amount, is done by this proceeding; and in the event of the inferences proving to be correct, we have at least gained an amount of time which is most valuable, the importance of which in fact can scarcely be over-estimated. We are not, as a general rule, long left in doubt as to the nature of the malady with which we have to deal, and if after a reasonable time symptoms definitely stamping the disease as anthrax have not shown themselves, we can discontinue our treatment and proceed in the manner indicated by the nature of the disease which has developed.

I am aware that the views here set forth will be condemned by many as empirical, but when it is considered that if we await the development

of the pathognomonic signs, the disease has arrived at a stage when curative measures are of little avail in a great majority of cases, they would appear to be worthy of some slight consideration. In further support of this line of argument another point may be brought forward, and that is the action of strong sunlight upon the spores. We are told that the action of this physical agent upon the spores is such that "it appears to be fatal, or at any rate injurious to them; it appears to deprive them in whole or in part of their powers of further development in a most remarkable manner, always causing distinct attenuation of their pathogenic virulence before completely destroying them.". On perusal of this statement it is brought forcibly before us that the conditions in India must in many cases lead to this attenuation of the virus, and thus a series of very much milder outbreaks of the disease result than is the case in some other countries. The fact that such a result is brought about has been impressed upon me during the course of my acquaintance with the disease in this country. I have had the opportunity of dealing with outbreaks in which the percentages of deaths have been so low as to lead to the supposition that the disease was not anthrax at all, and this despite the fact that at the commencement of the outbreak typical cases of a severe type, proving rapidly fatal, have occurred. It is in these outbreaks that the mode of treatment advocated has, I am assured, been the means of rescuing many horses from death.

The method adopted may be briefly noted. During an outbreak of anthrax horses of a troop or troops in which cases have occurred are carefully watched, and temperatures are recorded at least thrice daily. Animals suddenly showing a rise of temperature of some degrees are at once removed as suspects, and precautions as regards isolation are taken. They are at once subjected to an antiseptic treatment, which is so applied that average and frequently repeated doses of an antiseptic agent are administered, so as to ensure a continuous absorption of the drug and keep the system so far charged with it as is allowable. The choice of the antiseptic agent used may be left to the individual selection from the large numbers of these agents which are known. Under this system of treatment I have observed that the diagnostic symptoms of the disease, as we know it in India, have been slowly developed, and have been of far less intensity than in cases treated otherwise or not treated at all. The disease appears, in point of fact, to be more under control and to be of much shorter duration than under other circumstances.

This appears, then, to be the direction in which it is advisable to work, and it must be left to the practitioner to adopt the treatment which he considers most necessary to combat symptoms.

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